Exhibit A

GRANT & HACKH'S
CHEMICAL
DICTIONARY

[American, International, European and British Usage]

*Containing the Words Generally Used in Chemistry,
and Many of the Terms Used in the Related
Sciences of Physics, Medicine, Engineering,
Biology, Pharmacy, Astrophysics,
Agriculture, Mineralogy, etc.*

Based on Recent Scientific Literature

FIFTH EDITION
Completely Revised and Edited by

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The previous edition of this book was *Hackh's Chemical Dictionary*,
4th ed., published by McGraw-Hill in 1969. It was prepared by Dr.
Julius Grant from a *Chemical Dictionary* compiled by Ingo W. D.
Hackh. The current, or 5th, edition of this book was prepared by Dr.
Roger L. Grant, whose father prepared the 4th edition.

amodiaquine (hydrochloride) $C_{20}H_{22}ON_3Cl \cdot 2HCl \cdot 2H_2O$ = 464.8. Yellow, bitter crystals, m.158, soluble in water, an antimalarial (USP, BP).

amoeba Ameba.

amoeboid Resembling an ameba. **a. movement** The rolling movement of the protoplasmic jelly of a unicellular organism.

amoil Pentyl phthalate used for high-vacuum pumps.

amorphism The noncrystalline condition of a solid substance; due to an irregular molecular assembly.

amorphous (1) Unorganized. (2) Describing a solid substance which does not crystallize and is without definite geometrical shape. (3) In bacteriology, any bacteria without visible differentiation in structure.

amosite A long-fibered variety of Transvaal asbestos.

amoxy The pentyloxy* radical.

AMP* Symbol for adenosine 5′-monophosphate. See *adenylic acid*.

ampelite A graphite schist containing SiO_2 53.8, Al_2O_3 23.5%, and sulfur (Pyrenees). Used as refractory up to 1850°C.

Ampère, André Marie (1775–1836) French physicist; developed molecular theory.

ampere* A*. An SI base unit. The constant current that produces a force of 2×10^{-7} N/m length between 2 parallel conductors placed 1 m apart in a vacuum. 1 amp = 1 coulomb/s = 1 volt through 1 ohm = 10^{-1} emu (cgs) = $2 \cdot 998 \times 10^9$ esu. **micro ～** One-millionth of an a. **milli ～** One-thousandth of an a.

     a.meter Ammeter. **a.-volt** See *volt-ampere*.

amperometer Ammeter.

amperometry Chemical analysis by methods which involve measurements of electric currents. Cf. *conductometric analysis, polarograph, potentiometer*.

amphetamine $C_6H_5 \cdot CH_2 \cdot CH(NH_2)Me$ = 135.2. (±)-α-Methylphenethylamine, benzedrine, speed. Colorless liquid. Its vapors shrink nasal mucosa. **a. sulfate** $(C_9H_{13}N)_2 \cdot H_2SO_4$ = 368.5. Benzedrine sulfate. White, bitter powder; used for its stimulatory effects, particularly on nervous system; use is restricted owing to addictive nature.

amphi- Prefix (Greek) meaning "on both sides" or "both." **amphi position** Formerly, the 2,6-hydrogen atoms in 2 fused hexatomic rings; as, naphthalene.

amphiboles $M_x(SiO_3)_y$. Rock-forming minerals with occluded water; M is Ca, Mg, Fe, or the alkali metals, e.g., crocidolite. Elongated, fibrous, black or dark green crystals. Cf. *silica minerals*.

amphibolites Metamorphic rocks derived from argillaceous limestones and related to the glaucophane schists.

amphichroic, amphichromatic Describing mixed indicators whose color changes neutralize each other; e.g., litmus and congo red.

Amphicol Trademark for chloramphenicol.

amphigene Leucite.

amphiphatic Describing a detergent or wetting agent which greatly reduces the surface tension of water. Part of the molecule is hydrophilic, and the other consists of straight or branched long hydrocarbon chains; e.g., hexadecyltrimethyl bromide, $[C_{16}H_{33}Me_3N]^+Br^-$.

amphiphile A substance containing both polar, water-soluble and hydrophobic, water-insoluble groups; e.g., $C_{12}H_{25} \cdot OH$, where $C_{12}H_{25}$ is hydrophobic.

amphiprotic Able to lose or gain a proton. Cf. *acid, base*.

ampholyte Amphoteric electrolyte. A substance which in solution yields H^+ or OH^-, i.e., donates or accepts a proton, according to whether it is in an acid or basic solution. Cf. *isoelectric point, zwitterion*.

ampholytoid Amphoteric colloid. A particle in suspension capable of adsorbing H^+ or OH^-, depending on the pH value.

amphoteric Describing substances having both acid and basic properties; as, the amino acids, $NH_2 \cdot R \cdot COOH$. **a. hydroxides** The hydroxides of some metals which may dissociate to H^+ or OH^- ions; as, $2Al(OH)_3 \rightleftharpoons H^+ + AlO_2 + H_2O$ (acid) or $Al^{3+} + 3OH^-$(base). **a. sulfides** The sulfides of the metallic nonmetals, which may react as weak acids or weak bases; e.g., $H_3(AsS_3) \rightleftharpoons As(SH)_3$.

amphotericin B $C_{47}H_{73}O_{17}N$ = 924.1. Fungizone. A patented polyene antifungal antibiotic, produced by *Streptomyces nodosus*. Orange powder, insoluble in water (USP, BP).

amphotropine $[(CH_2)_6N_4]_2 \cdot C_8H_{14}(COOH)_2$ = 480. Hexamethylenamine camphorate. Colorless crystals.

ampicillin $C_{16}H_{19}O_4N_3S$ = 349.4. (6R)-6-(α-phenyl-D-glycylamino)penicillanic acid, Penbritin. White, bitter crystals, soluble in water; a broad-spectrum antibiotic, used for bacterial infections (USP, BP). Also used as the sodium salt and trihydrate (BP).

amplifier (1) A magnifier. (2) A transistor (or vacuum tube), by which weak electric currents or voltages are strengthened; as in radio reception.

amplitude The maximum displacement of an oscillation, vibration, or wave.

ampoule, ampul A small, sealed glass vial, e.g., sterilized solution for injection.

amrad gum A gummy exudation from elephant apple. *Feronia elephantum* (Rutaceae), of India.

amurca The bitter, watery residue from crushing olives; a pesticide.

amyctic (Greek: "to scratch") An irritating stimulant, especially for skin.

amygdala The seeds of *Prunus amygdalus*, containing amygdalin. **a. amara** See *bitter* under *almond*. **a. dulcis** See *sweet* under *almond*.

amygdalic acid (1) Mandelic acid. (2) $C_{20}H_{28}O_{13}$ = 476.4. Gentiobioside. A glucoside from almonds.

amygdalin $C_{20}H_{27}O_{11}N \cdot 3H_2O$ = 511.5. D-Mandelonitrile glucoside, amygdaloside. A glucoside from bitter almonds and wild cherry bark. Colorless crystals, m.210, soluble in water. Hydrolyzes to 2 moles glucose, hydrocyanic acid, and benzaldehyde; an expectorant and source of oil of bitter almonds. See *laetrile*.

amygdalinic acid Mandelic acid.

amygdaloid Shaped like an almond.

amygdophenin $EtOC_6H_4NH \cdot CO \cdot CHOH \cdot C_6H_5$ = 271.3. Phenetidine amygdalate. Gray crystals; an analgesic.

amyl The pentyl* radical, $Me(CH_2)_4-$. **iso ～** 3-Methylbutyl†. The isopentyl* radical, $Me_2CH(CH_2)_2-$. **tert- ～** The tert-pentyl* radical, $Et \cdot C(Me)_2-$.

     a.acetate Isopentyl *acetate**. **a.acetic ether** Isopentyl *acetate**. **a. alcohol** See *pentyl alcohol*. **a. hydride** Pentane*. **a. nitrite** (1) Pentyl nitrite*. (2) A mixture of nitrous acid, and 2- and 3-methyl butyl ester. Yellow volatile liquid, b.96. A vasodilator, used as inhalant and in angina, and as an antidote for cyanide poisoning (USP).

amylan A levorotatory gum in malt and barley; does not reduce Fehling's solution.

amylase* A group of enzymes that hydrolyze 1,4-α-D-glucosidic linkages. **α- ～ *** Diastase, ptyalase, ptyalin. Endohydrolyzes molecules containing 3 or more glucose units; e.g., starch to glucose, maltose and oligosaccharides. **β- ～ *** Diastase. Removes maltose units from nonreducing ends of chains; as, starch to maltose and dextrins. **gluco ～**

Exo-1,4-α-D-glu[...]
nonreducing en[...]
amylate A com[...]
amylene MeCl[...]
   1-Pentene*. is[...]
amylic alcohol[...]
amylidene The[...]
amyline The c[...]
amylo- Pertain[...]
amylobacter A[...]
   butanoic acid fei[...]
amylobarbital[...]
amylobarbitone[...]
amylocellulose[...]
   the starch granu[...]
amyloclastic A[...]
amylodextrin [...]
amyloform An[...]
   formaldehyde.[...]
amylogen A sol[...]
amylograph Ar[...]
   starch in terms o[...]
amyloid (1) (C_6[...]
   slightly soluble i[...]
   cellulose. Parchm[...]
   acid on cellulose[...]
   woody tissues.[...]
amyloin Maltod[...]
amylolysis The[...]
   dilute acids (hyd[...]
amylolytic Any[...]
   sugar. **a. activit**[...]
   enzyme Amylase[...]
amylopectin Th[...]
   75% of starch sul[...]
   structure. Its shor[...]
   laterally by α-(1,6[...]
amylose The sol[...]
   about 25% of star[...]
   chains of D-gluco[...]
   internally by α-(1[...]
amylum (1) Lati[...]
amyrin α- ～ Ili[...]
Amyris A genus[...]
   fragrant resins an[...]
   Mexico.[...]
Amytal Tradema[...]
an- Prefix indicat[...]
-an Suffix indicat[...]
   The systematic na[...]
   containing heteroi[...]
An Symbol for ac[...]
ana- Prefix (Gree[...]
   without, or agains[...]
   atoms attached to[...]
   hexatomic rings; a[...]
anabasine $C_{10}H_1$[...]
   isomer of nicotine[...]
   Central Africa. Co[...]
   water. (±)- ～ N[...]
anabolic (steroid)[...]
   anabolism, in part[...]
   muscles; as, andro[...]
anabolism Synth[...]
   of larger and more[...]
   ones; as, proteins [...]

Exhibit B

Hawley's

Condensed Chemical

Dictionary

THIRTEENTH EDITION

Revised by

Richard J. Lewis, Sr.

De

Sp

Derivation: By boiling an aqueous solution of ammonium cyanide with sulfur or polysulfides, or by the reaction of ammonia and carbon disulfide.

Grade: Technical, CP, 50–60% solution.

Use: Analytical chemistry; chemicals (thiourea); fertilizers; photography; ingredients of freezing solutions, especially liquid rocket propellants; fabric dyeing; zinc coating; weed killer and defoliant; adhesives; curing resins; pickling iron and steel; electroplating; temporary soil sterilizer; polymerization catalyst; separator of zirconium and hafnium, and of gold and iron.

ammonium thioglycolate. $HSCH_2COONH_4$.

Properties: Colorless liquid; repulsive odor. Evolves hydrogen sulfide. Combustible.

Use: Solutions of various strengths are used for hair waving and for hair removal.

ammonium thiosulfate.

CAS: 7783-18-8. $(NH_4)_2S_2O_3$.

Properties: White crystals decomposed by heat. PH of 60% solution 6.5–7.0. Very soluble in water.

Grade: Pure crystals (97%), 60% photographic solution.

Use: Photographic fixing agent, especially for rapid development; analytical reagent; fungicide; reducing agent; brightener in silver plating baths; cleaning compounds for zinc-base die-cast metals; hair waving preparations; fog screens.

ammonium titanium oxalate. (titanium ammonium oxalate). $(NH_3)_2TiO(C_2O_4)_2$.

Properties: A water-soluble powder

Use: Mordant in dyeing cellulosic fibers, leather, etc.

ammonium tungstate. (ammonium wolframate; ammonium paratungstate). $(NH_4)_6W_7O_{24} \cdot 6H_2O$.

Properties: White crystals. Soluble in water; insoluble in alcohol.

Derivation: Interaction of ammonium hydroxide and tungstic acid with subsequent crystallization.

Use: Preparation of ammonium phosphotungstate and tungsten alloys.

See ammonium metatungstate.

ammonium valerate. (pentanoic acid, ammonium salt; valeric acid, ammonium salt). $C_5H_{13}NO_2$.

Properties: Very hygroscopic crystals. Mp 108C, mw 119.16. Very soluble in water, alcohol, and ether.

Grade: Food and flavor codex.

Use: Flavoring material.

ammonium vanadate. See ammonium metavanadate.

ammonium wolframate. See ammonium tungstate.

ammonium zirconifluoride. See zirconium ammonium fluoride.

ammonium zirconyl carbonate.

$(NH_4)_3ZrOH(CO_3)_3 \cdot 2H_2O$. D 1.238 (24C). Stable up to approximately 68C; decomposes in dilute acids, alkalies.

Grade: Aqueous solution.

Use: Ingredient in water repellents for paper and textiles, catalyst, stabilizer in latex emulsion paints, ingredient in floor wax to aid in resistance to detergents, lubricant in fabrication of glass fibers.

ammonobasic mercuric chloride. See mercury, ammoniated.

ammonolysis. The procedure that is analogous to hydrolysis, with ammonia substituted for water.

"Ammo-Phos" *[Olin].* TM for high-analysis ammonium phosphate-containing fertilizers.

amniote egg. The type of egg laid by reptiles and birds, having a nutritious yolk and a hard outer shell to protect the embryo from the dry environment. The amniote egg is named for the amnion, a sac that contains the embryo.

amobarbital. (5-ethyl-5-isoamylbarbituric acid). $C_{11}N_{18}N_2O_3$.

Properties: White, crystalline powder; odorless; bitter taste. Mp 156–161C. Solutions are acid to litmus. Very slightly soluble in water; soluble in alcohol.

Grade: USP.

Hazard: May be a habit-forming drug of abuse.

Use: Medicine (also as sodium salt), hypnotic.

amodiaquine hydrochloride.

$C_{20}H_{22}ON_3Cl \cdot 2HCl \cdot 2H_2O$.

Properties: Yellow crystalline solid; odorless; bitter. Mp 150–160C (decomposes). Soluble in water; sparingly soluble in alcohol; very slightly soluble in benzene, chloroform, and ether; pH (1% solution) 4.0–4.8.

Grade: NF.

Use: Medicine (antimalarial).

amorphous. Noncrystalline, having no molecular lattice structure, which is characteristic of the solid state. All liquids are amorphous. Some materials that are apparently solid, such as glasses, or semisolid, such as some high polymers, rubber, and sulfur allotropes, also lack a definite crystal structure and a well-defined melting point. They are considered high-viscosity liquids. The cellulose molecule contains amorphous as well as crystalline areas. Carbon derived by thermal decomposition or partial combustion of coal, petroleum, and wood is amorphous (coke, carbon black, charcoal), though other forms (diamond, graphite) are crystalline. Amorphous metallic alloys for transformer coils are made by extremely rapid cooling of the molten mixture. They are composed of iron, nickel, phosphorus, and boron.

See liquid; liquid crystal; glass, metallic.

amorphous

amosite. A
See asbestos.

AMP. (1) .
propanol. (
phosphate.
See adenylic

A5MP. Ab
phoric acid
See 5'-adeny

"AMP-95"
propanol.
Grade: 95%
Available fo
Use: Multif
solubilizer,
lyst.

"Ampco" ;
num-iron-c
num, 1.5–!
fatigue, co
ting.
Use: For bu

"Ampcolo;
dustrial co
bronzes, i
manganes(
per, and hi

"Ampco-T
aluminum
rod conta
iron, bala:
metals an
corrosion,

AMPD. ;
propanedi

amphetam
methylphe
$C_6H_5CH_2($
Properties
strong od
(decompo
cohol and
Grade: De
phate and
Hazard: F
group of :
affect the
stricted tc
Use: Medi

Exhibit C

Amorphous pharmaceutical solids: preparation, characterization and stabilization

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Abstract

The importance of amorphous pharmaceutical solids lies in their useful properties, common occurrence, and physico-chemical instability relative to corresponding crystals. Some pharmaceuticals and excipients have a tendency to exist as amorphous solids, while others require deliberate prevention of crystallization to enter and remain in the amorphous state. Amorphous solids can be produced by common pharmaceutical processes, including melt quenching, freeze- and spray-drying, milling, wet granulation, and drying of solvated crystals. The characterization of amorphous solids reveals their structures, thermodynamic properties, and changes (crystallization and structural relaxation) in single- and multi-component systems. Current research in the stabilization of amorphous solids focuses on: (i) the stabilization of labile substances (e.g., proteins and peptides) during processing and storage using additives, (ii) the prevention of crystallization of the excipients that must remain amorphous for their intended functions, and (iii) the selection of appropriate storage conditions under which amorphous solids are stable. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Amorphous solid; Preparation of amorphous solid; Characterization of amorphous solid; Stabilization of amorphous solid

Contents

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1. Introduction

An amorphous solid (glass) can be defined with reference to a crystalline solid: similar to a crystalline solid, an amorphous solid may have short-range molecular order (i.e., in relationship to neighboring molecules); but unlike a crystalline solid, an amorphous solid has no long-range order of molecular packing or well-defined molecular conformation if the constituent molecules are conformationally flexible. Amorphous solids exist in many industrially important products, such as polymers, ceramics, metals, optical materials (glasses and fibers), foods, and pharmaceuticals. In the case of pharmaceutical materials, the importance of amorphous solids stems from:

1. Useful properties. Amorphous solids have higher solubility, higher dissolution rate, and sometimes better compression characteristics than corresponding crystals.
2. Instability. Amorphous solids are generally less stable physically and chemically than corresponding crystals.
3. Common occurrence. Amorphous solids can be produced by standard pharmaceutical processes and are the common form of certain materials (e.g., proteins, peptides, some sugars and polymers).

Although the amorphous solid has always been an essential part of pharmaceutical research, the current interest [1–3] has been elevated by two developments: (1) a growing attention to pharmaceutical solids in general, especially polymorphs and solvates [4–6] and (2) a revived interest in the science of glasses and the glass transition [7–9]. Studies of crystalline and amorphous solids are often so inter-twined that it is natural to treat the two solids as "polymorphs" of each other. This view is harmonious with one definition of polymorphism (i.e., any solids that share the same liquid state) [10], and with the "energy landscape" model of solids [11], which regards crystalline and amorphous states as connected minima on a multi-dimensional potential energy surface corresponding to different molecular packing, conformations, etc.

Since the study of amorphous solids has a long and rich history, it is appropriate to ask how pharmaceutical systems differ from other systems (polymers, ceramics, semi-conductors, optical glasses, etc.). From a functional standpoint, the overriding issues for pharmaceutical systems are the physico-chemical stability and bioavailability of the active ingredient, rather than such properties as mechanical strength and conductivity. From a structural standpoint, pharmaceutical systems often feature extensive hydrogen bonding, complex molecular geometry, and conformational flexibility. Such features make the problem of structural elucidation fundamentally different from, for example, that of inorganic glasses. The capacity to absorb water (hygroscopicity) and the ensuing consequences are of great concern to pharmaceutical systems. Furthermore, the stabilization of labile substances (e.g., proteins and peptides) is a distinctively pharmaceutical topic [12–14], with one objective being the prevention of structural damage during freezing and drying through the use of additives.

The topics discussed here — preparation, characterization, and stabilization of amorphous pharmaceutical solids — define a broad and active field, for which several excellent reviews have been published [1–3]. The aim of this review, therefore, is not to be comprehensive. In fact, topics well covered previously will be de-emphasized. Little will be said, for

example, about common experimental techniques, although the information derived from them is freely discussed. This should not be interpreted as a priority judgement and the reader should consult other reviews for relevant topics.

2. Preparation

For both thermodynamic and kinetic reasons, the preparation of amorphous solids is straightforward for some materials (good glass formers), but difficult for others (poor glass formers). Thermodynamically, the glass forming ability originates from a crystalline state that is not substantially more stable than the amorphous state, which may be the case for molecules that pack poorly or contain many internal degrees of freedom. Kinetically, a slow crystallization rate allows a material to become a "frozen liquid" or vitrify without crystallization.

One general cause for reduced crystallization tendency among organics is conformational flexibility [15]. Since conformationally flexible molecules can exist in a crystallizing medium as multiple conformers, the process of crystallization must select the "right" ones from among the "wrong" ones, a difficulty not encountered by rigid molecules. The effect is amplified if the conformers in crystals correspond to high-energy and low-concentration conformers in solution, which implies that the act of crystallization requires the average molecule to undergo a significant conformational change. The effect is believed to underlie the different crystallization tendencies of two stereoisomers, mannitol (easy) and sorbitol (difficult) [16,17]. In addition to conformational equilibria, configurational equilibria (e.g., that between carbohydrate anomers) should have similar effect on the tendency of crystallization. The effects of these equilibria on the glass-forming ability have not been well studied.

Poor glass formers (e.g., mannitol) can be made amorphous by deliberately preventing crystallization. Familiar routes to the amorphous state include quenching of melts, rapid precipitation by antisolvent addition, freeze-drying [12], spray-drying [18,19], and introduction of impurities [20]. The impurity effect may cause a poor glass former to exist in the amorphous state in a multi-component formulation.

Amorphous solids can also result from solid-dispersion [21], a process used to enhance bioavailability, and solid-state chemical reactions (e.g., degradation) of crystalline precursors.

Process conditions can influence the amount of amorphous materials in the end product. In a freeze-drying process, rapid freezing favors the formation of an amorphous solute, whereas introducing an annealing step may promote crystallization [12]. Processes that introduce mechanical or chemical stress (e.g., grinding, milling, and wet granulation) can render crystalline materials fully or partially amorphous. The concern over crystalline-to-amorphous conversion and the ensuing effects is amplified by the relative insensitivity of common techniques to small crystallinity changes (say, several %), but a generally strong dependence of the physicochemical stability of a product on the presence of amorphous materials. This concern has prompted the current interest in detecting amorphous solids at low levels.

Dehydration of crystalline hydrates has been demonstrated as a feasible and "gentle" route to the amorphous state of organic solids. Saleki-Gerhardt et al. showed that heating the crystalline raffinose pentahydrate at 60°C in vacuum converts the material to an amorphous form identical to one produced by freeze-drying [22]. Li et al. [23] observed that the crystalline carbamazepine dihydrate becomes amorphous upon dehydration at 45°C with N_2 purge. The resulting amorphous solid undergoes a glass transition at 56°C, which is significantly above the drying temperature (45°C), and crystallizes on further heating (at 86°C). These studies indicate that apart from being a potential route to amorphous solids, the drying of crystalline hydrates may reduce their physicochemical stability through the loss of crystallinity.

3. Characterization

The strategy for characterizing amorphous solids differs from that for crystalline solids. Molecular-level structural elucidation, as is feasible for crystalline solids by diffraction and spectroscopic methods, is less applicable to amorphous solids, and greater emphasis is placed on structural mobility and changes. It is customary to characterize an amor-

phous material both below and above the glass transition temperature, i.e., both as the frozen solid and as the supercooled viscous liquid. The physical characterization of amorphous solids utilizes a wide range of techniques (Table 1) and offers several types of information:

1. Structure. Amorphous solids are not random at the molecular level, but may possess short-range order, residual crystallinity, polymorphic states, and regions of different density.
2. Thermodynamics. Amorphous solids have higher energy, entropy and free energy than the corresponding crystals. The excess properties are parameters in some theoretical models of crystallization and structural relaxation.
3. Changes. Amorphous solids can crystallize or undergo structural relaxation owing to the instability with respect to the corresponding crystals and "equilibrium" glasses.
4. Multi-component systems. Many pharmaceutical formulations are multi-component, with water being a ubiquitous ingredient. It is desirable to predict properties of multi-component systems from those of individual components.

3.1. Structure

The structure of an amorphous solid is usually described as possessing crystal-like short-range mo-
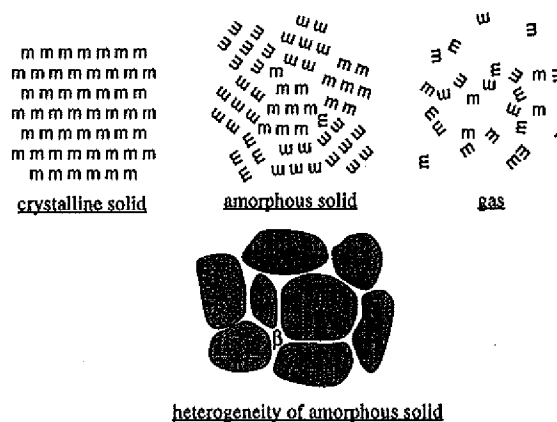
Fig. 1. Schematic representation of the structure of an amorphous solid. The molecular arrangement in an amorphous solid is not totally random, as in the gas phase, but features short-range molecular order similar to that in a crystalline solid. However, unlike crystals, an amorphous solid lacks the long-range order of molecular packing. According to some models, an amorphous solid has distinct regions (e.g., α and β) which have different densities and relaxation behaviors.

lecular arrangement, but lacking long-range order. As illustrated by Fig. 1, the immediate environment of a molecule (m) in an amorphous solid may not be significantly different from that in a crystal (e.g., similar number of and distance to the nearest neighbors), but an amorphous solid lacks any long-range translational-orientational symmetry that characterizes a crystal.

Table 1
Physical techniques for characterizing amorphous solids.[a]

Technique	Information
X-ray Diffraction (XRD)	DOC, CK
Molecular Spectroscopy	SR (e.g., Raman and NMR), microheterogeneity
Diff. Scan. Cal. (DSC)	DOC, microcrystalline or truly amorphous, CK, SR
Isothermal Calorimetry	SR, CK, DOC
Modulated DSC (MDSC)	Reversing vs. non-reversing heat flow, C_p, SR (0.1–0.01 Hz)
Solution Calorimetry	Excess enthalpy, DOC
Adiabatic Calorimetry	Excess enthalpy, entropy and free energy
Dielectric Analysis (DEA)	SR, primary vs. secondary processes
Dyn. Mech. Anal. (DMA)	SR
Viscometry	SR
Dilatometry	T_g, liquid/glass expansion coefficients
Solubility	Excess free energy
Density	Density difference from crystalline solids
Therm. Stimul. Cur. (TSC)	SR, DOC, microheterogeneity
Water Absorp. (gravimetric)	Hygroscopicity, DOC, CK

[a] Key: SR=structural relaxation (T_g, τ vs. T, fragility, etc.); DOC=degree of crystallinity; CK=crystallization kinetics.

3.1.1. Truly amorphous or microcrystalline

Grinding or milling of crystals can remove all traces of crystallinity according to XRD. Is the resulting material amorphous? Although successive micronization should eventually lead to an amorphous structure, a possibility exists that the material has achieved a *microcrystalline* state, containing crystals so small that they pass the detection of XRD. Johari et al. [24] used DSC to distinguish between amorphous and microcrystalline states based on the presence or absence of glass transition when XRD failed to do so.

3.1.2. Degree of crystallinity

Amorphous solids may co-exist with and have the potential to convert to crystalline solids. Techniques for determining the degree of crystallinity include XRD, DSC [25], solution calorimetry [26], water sorption [2], isothermal calorimetry [27], and thermally stimulated current (TSC) [28] (Table 1). Among these, water sorption [2] and TSC [29] are reported to provide greater sensitivity to low level amorphous solids. Isothermal calorimetry carried out in a vial-in-vial configuration is a popular technique for detecting crystallization in an amorphous sample. In this configuration, a sample is sealed in a vial along with a smaller vial containing a saturated salt solution, which provides an elevated humidity to accelerate crystallization.

3.1.3. Microheterogeneity

Dielectric studies of secondary relaxation in amorphous solids [30,31] advanced the view that a glass may have different regions: the glass transition (primary relaxation) involves cooperative motions in high-density regions, whereas secondary relaxation involves low-density regions lying between high-density regions (Fig. 1). Data from a recent TSC study have been interpreted as indicating the existence two amorphous regions (true and "rigid") in a drug sample [32].

Similarly, Tanaka [33] describes a supercooled liquid as having two competing tendencies of ordering: density ordering that leads to global crystallization and bond ordering that leads to locally favored structures whose packing and symmetry may differ from macroscopic crystals. In this model, locally favored structures are analogous to chemical im-

purities in that they can frustrate crystallization and influence the glass-forming ability.

3.1.4. Polyamorphism

The idea that there exist distinct amorphous phases separated by first-order phase transitions is a provocative one [34,35] It is unclear whether this phenomenon has any pharmaceutical relevance. However, the term polyamorphism has been used in a different way to describe amorphous states produced by different annealing times or preparative routes. An example is glasses that have been aged below T_g for different times and hence developed various degrees of "relaxation enthalpy". This should be considered an incorrect usage of the term, since these structures are related by structural relaxation (see later), not first-order transitions.

3.2. Thermodynamics

Thermodynamic properties of an amorphous solid often are presented as *excess properties* relative to the crystalline state (Fig. 2). Excess enthalpy, entropy and free energy can be obtained from heat capacities of the crystalline and amorphous phases as a function of temperature [36]. Excess enthalpy also can be obtained from heats of solution (by solution calorimetry) or crystallization (by scanning or isothermal calorimetry). In principle, excess free energy can be calculated from the solubility of crystalline and amorphous phases, provided that the equilibrium solubility of the amorphous solid can be measured without crystallization.

Excess thermodynamic properties are parameters in several theoretical models of structural changes in amorphous materials. The excess free energy is a parameter in the classical theory of nucleation, which along with surface tension gives the work necessary to form a nucleus of critical size [37]. The excess entropy enters the Adam-Gibbs model of structural relaxation [38], giving the dynamic behavior a thermodynamic underpinning.

3.3. Changes

3.3.1. The glass transition temperature

If crystallization is avoided, many liquids of pharmaceutically relevance vitrify at a temperature (the glass temperature, T_g) approximately 2/3 to 4/5
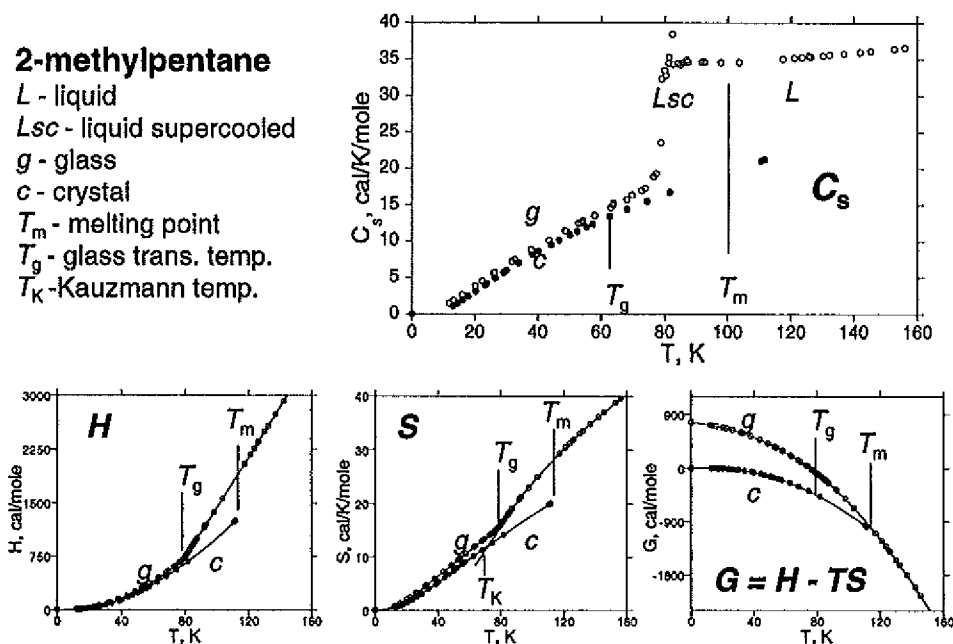
Fig. 2. Thermodynamic properties of the crystalline and amorphous phases of 2-methylpentane. The heat capacity (C_s) data (Douslin, D. R.; Huffman, H. M. *J. Am. Chem. Soc.*1946, *68*, 173) have been integrated to give the enthalpy (H), entropy (S), and free energy (G). Excess properties correspond to the difference between the crystalline and amorphous lines. The "entropy crisis" of Kauzmann (Kauzmann, W. *Chem. Rev.*1948, *43*, 219–256) is seen in the *S* panel as the impending crossing of the liquid line with the crystal line as the temperature decreases below the crystal melting point T_m. If not for the intervention of the glass transition at T_g, the liquid line would have crossed the crystal line at T_K. This would be an absurdity, since the liquid entropy would be lower than crystal entropy below T_K.

of the crystalline melting point T_m measured in Kelvin [3,39]. Unlike T_m, T_g is a kinetic parameter, depending on temperature scanning rate and thermal history. Nonetheless, T_g is a useful material descriptor owing to its correlation with structural and thermodynamic properties.

Although numerous material properties (heat capacity, volume, dielectric relaxation, etc.) can be used for T_g measurement, DSC has recently become a principal source of T_g data for many types of materials, including pharmaceuticals [3]. Quantitative measurement of T_g takes into account the effect of impurities (often water), scanning rate, and annealing, and distinguishes between onset, midpoint, and endpoint temperatures (Fig. 3). In more sophisticated analyses, the limiting fictive temperature T_f' is calculated [40], which gives the "true" liquid-glass crossing point of the enthalpy-temperature curves that is independent of scanning rate. Thus, T_f' obtained upon cooling from well above the glass transition region depends solely on the material.

However, the measurement of cooling T_f' may be confounded by thermal degradation and lower definition of the glass transition as compared to the measurement of T_g by heating.

Modulated DSC [41] can be used to separate the reversing and non-reversing components of a glass transition (e.g., in spray-dried lactose [42]), a beneficial utility in the assignment of glass transitions that are weak or overlap with other thermal events (Fig. 3). It is of interest to obtain T_g of poor glass formers (e.g., mannitol and glycine), because of its use in estimating T_g of mixtures in which the poor glass former remains amorphous. Apart from quench cooling, a melt-miscible impurity may be introduced to inhibit crystallization [43].

3.3.2. Crystallization

If a more stable crystalline state exists, an amorphous material can crystallize when sufficient molecular mobility exists. Pharmaceutically important examples include crystallization in freeze- and spray-
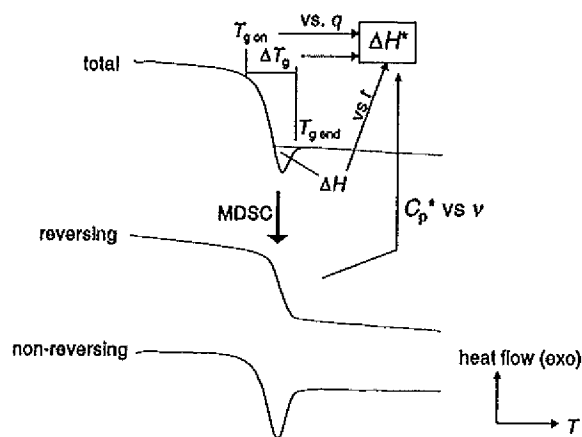
Fig. 3. Illustration of the uses of DSC data for measuring T_g and ΔH^* (the activation energy for enthalpy relaxation). $T_{g\,on}$, $T_{g\,end}$, and ΔT_g indicate the onset, end, and width of the glass transition. Modulated DSC (MDSC) allows the separation of the total heat flow into reversing and non-reversing components. ΔH^* can be evaluated from (i) the dependence of $T_{g\,on}$ on scanning rate q, (ii) ΔT_g, (iii) the dependence of the "relaxation enthalpy" ΔH (area of the "overshoot") on annealing time, and (iv) the dependence of the complex heat capacity C_p^* (obtainable by MDSC) on modulation frequency ν. See text for details.

drying, from supercooled melts, and from amorphous materials during storage, especially on exposure to heat and humidity. Of interest in this context are factors affecting the rate of crystallization (e.g., temperature and plasticizers), means to promote or prevent crystallization, and the characteristics of crystals produced under conditions unfavorable for growing "high quality" crystals (e.g., the high-concentration and high-viscosity media encountered in freeze- or spray-drying).

Crystallization of carbohydrates and derivatives, which are common pharmaceutical excipients, presents special challenges. Elusive crystallization behaviors of such compounds are familiar to carbohydrate researchers. Xylitol, discovered as a syrup, crystallized initially as a metastable polymorph, then as a more stable polymorph, with the metastable form being impossible to make again [44]. The crystallization of D-glucose is influenced by the presence of α (36%) and β (64%) anomers in solution in so-called mutarotational equilibrium [45]. Although the β-anomer is more stable in solution, the commercial crystal form contains the α-anomer only. A similar situation exists for lactose [46],

which in solution takes on two anomeric forms, α (38%) and β (62%), but crystallizes normally as an α-anomer monohydrate from water. A β-anhydrate precipitates from concentrated solutions above 93.5°C. Crystallization of amorphous lactose produced by spray-drying (24% α and 76% β) produces a crystalline mixture of α-monohydrate and β-anhydrate containing 29% α [47]. Mannitol and sorbitol, two isomers with different stereochemistry on only one carbon, have significantly different tendencies to crystallize, which have been attributed to whether or not a major conformational change is required on crystallization [16,17].

The existence of multiple crystal forms, as shown by spray-dried lactose [47], further complicates the crystallization of amorphous solids. Mannitol [20,48–59], sorbitol [60], dulcitol [61], lactose [46], and trehalose [62] all show polymorphism and/or hydrate formation. Crystallization of mannitol from freeze-concentrated solutions can yield pure polymorphs a hydrate [59] polymorphic mixtures, or crystalline-amorphous mixtures, depending on concentration, processing conditions, and the presence of other ingredients [63–66]. It is unclear whether different anhydrous polymorphs of mannitol can significantly impact the properties of a freeze-dried product. However, the difference between anhydrous and hydrated crystals or between crystalline and amorphous solids is likely to cause pronounced differences in product performance. For example, the formation of a mannitol hydrate during freeze-drying may retain more water in the product, which may be subsequently released (e.g., at a high storage temperature), causing accelerated degradation of the active component [59].

The nucleation-growth model recognizes two distinct steps in crystallization that have different temperature dependence: lower temperature favors nucleation and higher temperature favors growth [67]. As a result, maximum crystallization rate occurs between preferred temperatures of nucleation and growth. In the case of indomethacin crystallizing from the amorphous state, the α polymorph has a maximum nucleation rate at 60°C and a maximum growth rate at 90°C [68]. The nucleation-growth model also provides a practical guide for controlling crystallization. For example, it is a familiar DSC observation that many supercooled liquids do not

crystallize during cooling, but do so upon reheating, often soon after passing T'_g. Cooling a 10% w/v mannitol solution in water can cause partial solute crystallization (after ice crystallization). However, even at a slow cooling rate (say, 0.5°C/min), crystallization of mannitol is incomplete in the cooling step and additional crystallization occurs during reheating (near −25°C). This crystallization can be so violent as to break freeze-drying vials [69,70]. These phenomena are explained by the efficient nucleation at low temperature and the subsequent growth of these nuclei to mature crystals at higher temperatures.

Cooling rate also affects the rate of nucleation [71]. Slow cooling allows the maintenance of a steady-state nucleation rate, whereas rapid cooling prevents a full development of viable nuclei. As a result, rapid cooling not only facilitates glass formation but also enhances glass stability against crystallization. However, a rapid entry into the glassy state may give rise to another instability, that with respect of structural relaxation (see later). The balance between these two types of stability illustrates the dimensions to be explored in the optimization of material properties.

A method for studying the nucleation effect on crystallization is two-step DSC [72]. As Fig. 4

shows, the temperature dependence of the crystallization rate is evaluated first by cooling the system from an equilibrated liquid state to different crystallization temperatures, T_c (so-called one-step experiment). For a system following a nucleation-growth mechanism, the one-step experiment will produce the temperature of maximum crystallization rate, $T_{c\ max}$. In the two-step experiment, the system is cooled from the same initial liquid state to a temperature $T_n < T_{c\ max}$ and then returned to $T_{c\ max}$. If there is a nucleation effect, the two-step crystallization rate will be significantly faster than the one-step crystallization rate. In the example shown, the crystallization of sorbitol spherulites from a supercooled melt was measured. The maximum one-step crystallization rate occurs at approximately 40°C. By briefly exposing the sample to $T_n = 20$°C, where the sample has negligible crystallization, the crystallization rate at 40°C becomes significantly faster.

3.3.3. Structural relaxation

When a material is isolated in a metastable crystalline state, it may behave as if it is independent from the stable crystal form, until a "catastrophic" first-order polymorphic transition takes place. An amorphous solid, on the other hand, may behave as if it always "recognizes" the presence of the more

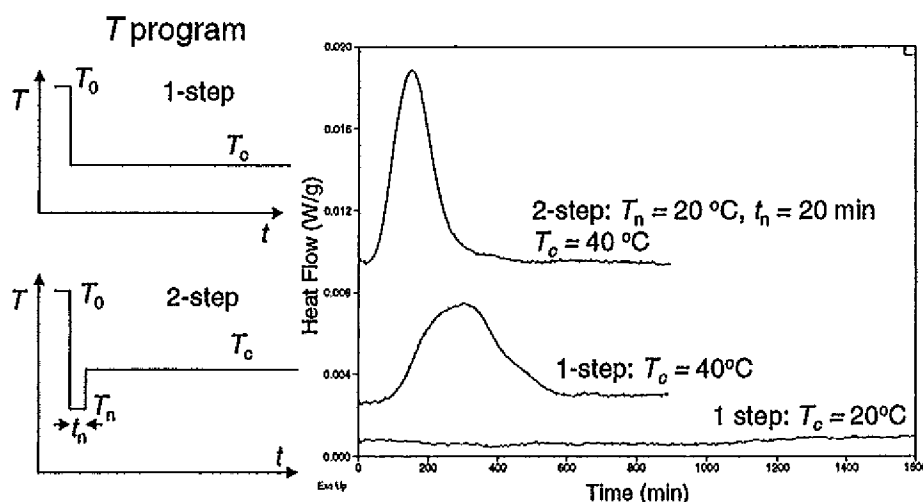Fig. 4. An illustration of the two-step DSC experiment for investigating the effect of nucleation on crystallization. If lower temperature causes faster nucleation, results similar to what is shown here may be observed: the total crystallization rate observed in the two-step experiment (T_c followed by T_n) may be significantly faster than that observed in one-step experiments conducted at either T_c or T_n.

stable equilibrium glassy state and continuously evolves towards it in a manner predictable from its thermal history and the degree of non-equilibrium. This process is known as structural relaxation, physical aging, or annealing.

If structural relaxation occurs exponentially, a characteristic time, τ, can be defined, which is a measure of the "mobility" in the material. Structural relaxation can be studied on different time scales by following the time evolution or frequency dependence of many material properties: enthalpy, volume, viscosity, shear modulus [73], dipole relaxation, depolarization current [74], and nuclear spin relaxation [75] (Table 1). The τ vs. T (temperature) data thus obtained are usually plotted as $\ln \tau$ vs. $1/T$, the slope of which gives the activation enthalpy of structural relaxation $\Delta H^* \; [= -Rd \ln \tau/d(1/T)]$.

When the property measured is enthalpy, DSC can be used to characterize structural relaxation, now called enthalpy relaxation (Fig. 3). T_g vs. scanning rate (q) data can be used to determine ΔH^* [76]. ΔH^* obtained in this way is found indistinguishable from ΔH^* of viscous flow [77]. T_g used for this analysis can be obtained in two ways: (1) $T_{g \; onset}$ measured at different heating rates q_h after cooling at the same rates ($q_h = q_c$) without annealing; (2) limiting fictive temperature T_f' recorded (at any fixed heating rate, say 10°C/min) after cooling at different rates q_c without annealing. An advantage of Method (2) over Method (1) is that Method (2) does not require absolute temperature calibration at each scanning rate [40]. In principle, the cooling T_g can also be used in this analysis. However, it is necessary to perform absolute temperature calibration during cooling, a non-trivial task since most first-order transitions used for DSC calibration (e.g., indium melting) show significant supercooling. For temperature calibration in the cooling mode, low-energy transitions in liquid crystals are useful [78,79].

Moynihan [80] observed that the width of glass transitions correlates with the activation energy ΔH^* in the form $(\Delta H^*/R)(1/T_{g \; on} - 1/T_{g \; end}) = C = 4.85$ for a group of high-T_g inorganic glasses. Bruning and Sutton [81] reached a similar conclusion by comparing two dimensionless parameters: $T_g(d^2 T_f/dT^2)|_{T=T_g}$ for width and $-T_g(d \ln \eta/dT)|_{T=T_g}$ for activation energy.

Other DSC-based techniques have been applied to study the structural relaxation in amorphous systems. The use of modulated DSC (MDSC) can determine the frequency dependence of the complex heat capacity and probe structural relaxation at a relatively low frequency (0.1–0.01 Hz) [82]. Isothermal DSC has been used to study sub-T_g enthalpy recovery in ethylene–water solution [83] and in glycerol and propylene glycol [84]. The effect of isothermal annealing was measured relative to non-annealed control to determine the temperature and time dependence of enthalpy relaxation. The time-evolution of the excess enthalpy has been measured by tracking the size of the DSC enthalpy relaxation peak as a function of sub-T_g annealing time for sucrose, PVP and indomethacin [85], as well as for binary systems containing sucrose [86]. These data reveal a non-Arrhenius character of enthalpy relaxation in these systems.

Fragility. The idea of fragility [87] originates from plotting the structural relaxation time τ in the Arrhenius form with the temperature scaled by T_g, i.e., log τ vs. T_g/T. In this plot (the "Angell plot"), materials of many types intersect at $T_g/T = 1$ with $\tau = 10^2$ s. Furthermore, some materials, called strong, show quasi-Arrhenius behavior (log τ linear in T_g/T), whereas others, called fragile, deviate significantly from the Arrhenius behavior. The strong–fragile pattern observed in this plot is well reproduced by Eq. (1):

$$\tau = \tau_0 \exp[DT_0/(T - T_0)] \tag{1}$$

In Eq. (1), the "strength parameter" D describes the deviation from the Arrhenius behavior, with strong systems featuring $D > 25$ and fragile systems $D < 10$ (Fig. 5). The parameter T_0 is sometimes called the temperature of "zero" mobility and is found to correlate with D in the approximate form $T_g/T_0 = 1 + D/39.1$.

As the temperature scale of Fig. 5 indicates, the idea of fragility applies to supercooled liquids near and above T_g. Even so, the fragility classification is meaningful to amorphous solids because it is near the T_g when rate processes become more important in real time. In other words, fragility indicates how fast structural relaxation accelerates as a glass approaches and traverses the glass transition region.
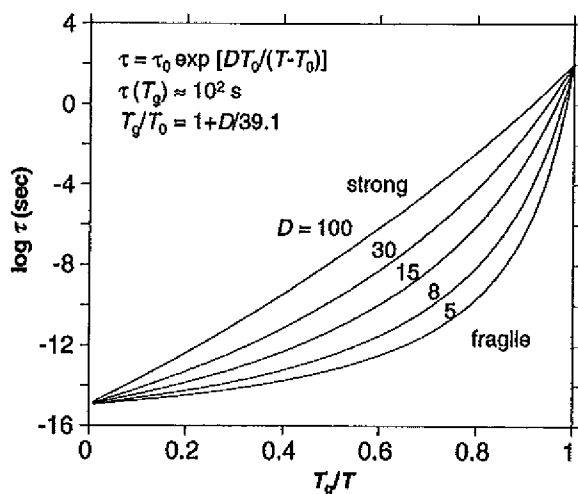
Fig. 5. The strong–fragile pattern that characterizes the temperature dependence of structural relaxation times of supercooled liquids. The series of curves are generated by Eq. (1) using different D values and give a good reproduction of the pattern that emerges from the "Angell plots" (log τ vs. T_g/T) of many liquids. Strong liquids (e.g., SiO_2) are characterized by large D and quasi-Arrhenius behavior and fragile liquids (e.g., many small-molecule organics) by small D and non-Arrhenius behavior.

This characteristic is not captured by T_g, which, in fact, is independent of fragility [81].

The empirical fragile–strong pattern has been given a thermodynamic basis [87] through the Adam–Gibbs model [38], in which the rate of structural relaxation is linked to the excess entropy in the amorphous state. A key feature of the linkage is the identity between T_0 in Eq. (1) (from kinetic measurements) and the Kauzmann temperature T_K (from thermodynamic measurements). Fragility has also been correlated with features of the energy "landscape" [87] and the non-exponential character of structural relaxation [88].

The success of the fragility concept has prompted searches for the structural basis of fragile and strong behaviors [87,81]. Examples of strong glasses are given by materials with self-reinforcing network structures (e.g., SiO_2) and certain proteins [89,90]. Systems containing non-directional intermolecular/interatomic bonds and internal flexibility tend to be fragile. Small-molecule organics often are found in the fragile category.

Although the qualitative meaning of the strong/

fragile behavior is well accepted, how fragility should be quantified is still being defined. The strength parameter D is obtained by fitting viscosity or dielectric relaxation data to Eq. (1) [87].. The disadvantages of the D metric are that the Eq. (1) behavior is not always followed and that D approaches infinity as T_0 approaches 0 K, resulting in a mathematical inconvenience [91]. The steepness parameter m, defined by $m=(T_g)^{-1}d \ln\tau/d(1/T)|_{T=T_g}$, eliminates model dependence and can be determined from the activation energy for structural relaxation ΔH^* (see above). With this metric, strong systems feature $m<40$ and fragile systems $m>75$. The $F_{1/2}$ metric is defined in terms of T_g and the temperature $T_{1/2}$ at which structural relaxation time equals 10^{-6} s: $F_{1/2}=2[T_g/T_{1/2}-0.5]$ [92]. The value of $F_{1/2}$ lies between 0 (strong) and 1 (fragile). $F_{1/2}$ is also model independent and compared to m, a more robust quantity, since material properties change rapidly near T_g, where m is measured, causing larger experimental errors. Angell proposed a procedure for measuring $F_{1/2}$ based on a DTA measurement in the presence of a $10^{5.2}$ Hz oscillating electric field, which provides T_g and $T_{1/2}$ from a single scan [93]. The width of glass transition has also been used to assess fragility [20,80,81]. This correlation was recently substantiated by comparing to the $F_{1/2}$ metric and used to justify the strong behavior of water in the glass transition region (ca. 136 K) and a fragile-to-strong transition in water [94]. Hancock et al., however, report that the width method requires careful "calibration" for general applications [95].

Primary (α) vs. secondary (β) processes. In addition to the glass transition, the so-called primary or α process, many glasses (polymeric and small-molecule) exhibit a secondary or β process [24,30]. Although secondary relaxations are generally weak, and can weaken with annealing, some pharmaceutical materials, e.g., sorbitol [96,97], exhibit surprisingly strong secondary relaxation. The α-process is usually described as general, cooperative, non-Arrhenius, linked to viscous flow, and synonymous with the glass transition, whereas the β-process as specific, local, Arrhenius, and of molecular origin. Hikima et al. proposed that the crystal growth rate in triphenylethylene near T_g is controlled by the β process, rather than the α process [98].

3.4. Multi-component systems

A central question concerning multi-component systems, to which most pharmaceutical formulations belong, is whether the structures, thermodynamics, and changes can be predicted from the properties of its components. For example, how do additives affect the rates of crystallization and structural relaxation? What is the effect of water absorption on amorphous solids? Since sugars and sugar alcohols are commonly used to stabilize proteins and peptides (Section 4), what are the behaviors of these solid systems of small- and large-molecule components?

In the case of T_g, several equations have been introduced to link the T_g of a mixture to the T_g's of its components, including Fox [99], Couchman [100], and Gordon-Taylor [101]. Which equation performs better has not been firmly established and in certain circumstances, the difference in performance is marginal. Since the nature of interactions between pharmaceutical components varies greatly, depending on molecular size and ionic state, it is unlikely that any equation applies universally. The plasticizing effect of water has been modeled successfully using a simplified Gordon-Taylor equation [102].

The crystallization of amorphous indomethacin can be inhibited using low-level polymeric additives [poly(vinylpyrrolidone) and poly(vinylpyrrolidone-co-vinylacetate)] [103]. The effect of additives on the structural relaxation in sucrose has been examined using the ΔH (overshoot) technique (Fig. 3). This type of study may be beneficial to the understanding of protein–carbohydrate formulations.

The special importance of water has prompted studies of its effect on amorphous pharmaceutical solids. Apart from plasticization, water can accelerate chemical degradation and crystallization. Shalaev and Zografi [104] considered scenarios in which water can affect chemical degradation in amorphous materials: as reactant, product, medium, or plasticizer. Water can affect the crystallization of amorphous solids both as a plasticizer to enhance structural mobility and as a building unit of hydrated crystals. The latter scenario is relevant to excipients that can crystallize as hydrates: lactose (monohydrate), trehalose (dihydrate), glucose (monohydrate), and mannitol (stoichiometry unknown).

4. Stabilization

Research aimed at stabilizing amorphous solids is multi-faceted, including: (i) the stabilization of labile biomolecules (e.g., proteins and peptides) through additives, (ii) the prevention of crystallization of excipients that must remain amorphous for their intended functions, (iii) the specification of appropriate storage temperatures to achieve acceptable shelf life, and (iv) the prevention of chemical degradation and microbial growth through anti-oxidant, pH buffer, preservatives, etc. Our discussion focuses on the "physical" aspects of stabilization (i–iii). Lai and Topp have reviewed chemical degradation pathways common to proteins and peptides in the solid state [105].

4.1. Stabilization of labile biomolecules

Freezing and drying are essential steps in the preparation of protein and peptide formulations [12] and in the preservation of organisms [14]. Such treatments can be detrimental to these naturally hydrated species. It has been observed that the proteins, peptides and organisms can be effectively protected against freezing and drying damages when they are co-processed with certain excipients, typically carbohydrates and derivatives (sucrose, trehalose, mannitol, sorbitol, etc.) [12–14,106]. Although the mechanism of stabilization is not firmly established, it is thought to involve both vitrification and direct interactions.

4.2. Vitrification

Vitrification-based stabilization relies on the immobilization and isolation of labile substances in rigid glasses of inert stabilizer molecules. Vitrification is expected to reduce the potential for protein aggregation and diffusion of small molecules required to initiate hydrolysis, oxidation, etc. [105] The general assessment of the vitrification hypothesis seems to be that vitrification is necessary but insufficient for stabilizing labile substances and that direct (specific) interactions also are required [14].

In vitrification-based stabilization strategies, T_g provides a concrete guide to the selection of stabilizers and storage temperatures. By eliminating plas-

ticizers (e.g., water) and introducting antiplasticizers, one increases T_g and reduces structural mobility. Shamblin and Zografi [85] showed that antiplasticizers effectively reduce structural mobility in amorphous sucrose. A more sophisticated analysis takes into account of both T_g and fragility, using the "zero-mobility" temperature T_0 as the parameter for ranking the relative stability of potential formulations [107].

4.3. Direct or specific interactions

Besides vitrification, direct drug-excipient interactions are important for stabilization [14]. An example of such interactions is the selective hydrogen bonding between stabilizing excipients and the drug molecules. These interactions may resemble the way in which water molecules are integrated into the structures of proteins and peptides (the water-replacement hypothesis).

A well developed concept is that conformational change of proteins during freeze-drying is generally detrimental and should be avoided [13]. This is a sound strategy so long as the conformational change is irreversible. In the crystallization of carbohydrates and other small-molecule organics [108], conformational changes upon solidification are common, but often reversible upon dissolution. If such cases, conformational changes on freezing and drying would not be indicative of structural damage.

4.4. Protection against crystallization of stabilizing excipients

It is generally accepted that in order to act as stabilizers, an excipient must mix homogeneously with the drug to be stabilized [12]. However, certain excipients (e.g., mannitol) have strong tendency to crystallize, leading to phase separation and loss of stabilizing power. Crystallization can also lead to the formation of slow dissolving particles, causing slow reconstitution of parenteral products.

Despite potential crystallization problems, excipients with strong tendency to crystallize can sometimes make suitable stabilizers. In the case of mannitol, its crystallization tendency is compensated by a superior chemical stability against oxidation and hydrolysis in comparison to disaccharides. For example, mannitol is stable at low or high pH where disaccarides undergo hydrolysis. Amorphous sucrose can undergo acid-catalyzed inversion even at very low levels of residual water [109]. In some cases [63,66], the "flaw" of mannitol as a poor glass former can be remedied by proteins and peptides themselves, which effectively inhibit crystallization.

4.5. The T_0 or $T_g - 50$ K rule

Molecular mobility that allows physical aging and crystallization [110] of glasses below T_g implies that T_g is unsatisfactory as an indicator for the temperature below which molecular motions "cease" for practical purposes. If structural relaxation follows Eq. (1), then the parameter T_0 represents the temperature at which the relaxation time τ goes to infinity ("zero" mobility). It has been proposed [2,111] that T_0, rather than T_g, be used as a practical guide for selecting storage temperatures. For many fragile glasses, T_0 is approximately 50 K below T_g.

The $T_g - 50$ K rule is an important reminder of the finite structural mobility below T_g. This rule, of course, is dependent on several conditions: fragile systems, Eq. (1) behavior, and α-relaxation process. With strong materials, T_0 will lie significantly below $T_g - 50$ K. For materials that deviate from Eq. (1), the T_0 parameter becomes irrelevant. Finally, even though it is plausible that structural changes required for crystallization and chemical degradation correlate with the cooperative α-process, it has been suggested that the β-process also may regulate the crystallization process [98].

4.6. Trehalose

Trehalose has achieved a special status among stabilizing excipients, even though its superiority is unproven [112]. Comparison has been made between trehalose and sucrose, for example, to understand their potential difference in stabilizing ability. Trehalose exists commonly as a dihydrate and has anhydrous polymorphs [62], whereas sucrose exists as anhydrate (with some hygroscopicity) and is not known to be polymorphic. Trehalose has higher T_g [112] and is more fragile [111,113], both in the dry state and in aqueous solutions, than sucrose. Trehalose is believed to have a greater "destructuring"

effect on the water structure, thus preventing ice formation, than sucrose and maltose [114]. Such differences have been used to argue for the effectiveness of trehalose as a stabilizer: higher T_g lends rigidity to the matrix, fragility and polymorphism make the matrix more "adaptable" to guest molecules, high T_g and high fragility lead to high T_0 (temperature of "zero" mobility), the "destructuring" effect makes trehalose a better anti-freezing agent, and the ability of forming a hydrate "sequesters" moisture otherwise available for chemical degradation.

Although these arguments are persuasive for this pair of sugars, the question about the uniqueness of trehalose has not been satisfactorily answered. In every respect that trehalose is "superior" to sucrose, other compounds exist that match or surpass trehalose. It is also unclear whether the chemical stability of trehalose (a non-reducing sugar) is important to its stabilizing function.

5. Concluding remarks

Amorphous solids exist widely in and impart special properties to pharmaceutical products. This review has examined concepts and approaches that are relevant to the preparation, characterization and stabilization of amorphous pharmaceutical solids. What can we extrapolate from the present state of affairs? The recognition of broad patterns of structural relaxation dynamics justifies searches for similar patterns shown by rate processes of greater complexity and importance: crystallization and chemical degradation. Given their instability, general strategies for stabilizing amorphous solids against crystallization and structural relaxation would be desirable. Continuing studies on the stabilization of labile biomolecules should benefit from a better knowledge of the relative importance of vitrification and direct interaction. General behaviors of multi-component systems deserve attention. Amorphous solids prepared by unconventional routes (e.g., drying of crystalline hydrates) are of interest for understanding the structural and relaxational aspects of the glassy state. The nature of crystallization in amorphous solids, as it differs from that in dilute solutions, warrants attention, as do the effects of annealing and

conformational and configurational complexity of organic molecules.

References

[1] D.Q.M. Craig, P.G. Royall, V.L. Kett, M.L. Hopton, The relevance of the amorphous state to pharmaceutical dosage forms: glassy drugs and freeze dried systems, Int. J. Pharmaceut. 179 (1999) 179–207.

[2] B.C. Hancock, G. Zografi, Characteristics and significance of the amorphous state in pharmaceutical systems, J. Pharm. Sci. 86 (1997) 1–12.

[3] J. Kerč, S. Srčič, Thermal analysis of glassy pharmaceuticals, Themochim. Acta 248 (1995) 81–95.

[4] T.L. Trelfall, Analysis of organic polymorphs: a review, Analyst 120 (1995) 2435–2460.

[5] H.G. Brittain (Ed.), Polymorphism in Pharmaceutical Solids, Marcel Dekker, New York, 1999.

[6] L. Yu, S.M. Reutzel, G.A. Stephenson, Physical characterization of polymorphic drugs: an integrated characterization strategy, Pharm. Sci. Tech. Today 1 (1998) 118–127.

[7] C.A. Angell, Formation of glasses from liquids and biopolymers, Science 267 (1995) 1924–1935.

[8] A broad recent interest in glasses and the glass transition has resulted in several special issues: J. Non-Crystalline Solids, 131–133(1991); Science 267(1995); ACS Symposium Series 676 (Supercooled liquids: advances and novel applications); and J. Phys. Chem. 103(1999) (dedicated to C. A. Angell).

[9] M.D. Ediger, C.A. Angell, S.R. Nagel, Supercooled liquids and glasses, J. Phys. Chem. 100 (1996) 13200–13212.

[10] J. Haleblian, W. McCrone, Pharmaceutical applications of polymorphism, J. Pharm. Sci. 58 (1969) 911–929.

[11] F.H. Stillinger, T.A. Weber, Packing structures and transitions in liquids and solids, Science 225 (1984) 983–989.

[12] M. Pikal, Freeze-drying of proteins: process, formulation and stability, in: L.J. Cleland, R. Langer (Eds.), ACS Symposium Series 567, Formulation and Delivery of Proteins and Peptides, ACS, Washington, DC, 1994, pp. 20–133.

[13] J.F. Carpenter, Interactions of stabilizers with proteins during freezing and drying, in: L.J. Cleland, R. Langer (Eds.), ACS Symposium Series 567, Formulation and delivery of proteins and peptides, ACS, Washington, DC, 1994, pp. 134–147.

[14] J.H. Crowe, J.F. Carpenter, L.M. Crowe, The role of vitrification in anhydrobiosis, Annu. Rev. Physiol. 60 (1998) 73–103.

[15] L. Yu, S.M. Reutzel-Edens, C.A. Mitchell, Crystallization and polymorphism of conformationally flexible molecules: problems, patterns, and strategies, Org. Proc. Res. and Dev. 4 (2000) 396–402.

[16] M. Siniti, S. Jabrane, J.M. Letoffe, Study of the respective binary phase diagrams of sorbitol with mannitol, maltitol and water, Thermochim. Acta 325 (1999) 171–180.

[17] G.A. Jeffrey, H.S. Kim, Conformations of the alditols, Carbohydr. Res. 14 (1970) 207–216.

[18] J. Broadhead, S.K. Rouan Edmond, C.T. Rhodes, The spray

drying of pharmaceuticals, Drug Dev. Ind. Pharm. 18 (1992) 1169–1206.

[19] R.W. Niven, Delivery of biotherapeutics by inhalation aerosols, Pharm. Technol. 17 (1993) 72–82.

[20] L. Yu, D.S. Mishra, D.R. Rigsbee, Determination of the glass properties of D-mannitol using sorbitol as an impurity, J. Pharm. Sci. 87 (1998) 774–777.

[21] W.L. Chiou, S. Riegelman, Pharmaceutical applications of solid dispersion systems, J. Pharm. Sci. 60 (1971) 1281–1302.

[22] A. Saleki-Gerhardt, J.G. Stowell, S.R. Byrn, G. Zografi, Hydration and dehydration of crystalline and amorphous forms of raffinose, J. Pharm. Sci. 84 (1995) 318–323.

[23] Y. Li, J. Han, G.G.Z. Zhang, D.J.W. Grant, R. Suryanarayanan, In situ dehydration of carbamazepine dihydrate: a novel technique to prepare amorphous anhydrous carbamazepine. Pharmaceutical Development and Technlogy (in press).

[24] G.P. Johari, S. Ram, G. Astl, E. Mayer, Characterizing amorphous and microcrystalline solids by calorimetry, J. Non-Crystalline Solids 116 (1990) 282–285.

[25] B. Cassel, B. Twombly, Rapid DSC determination of polymer crystallinity, Am. Lab (Jan.(1998)) 24–27.

[26] M.J. Pikal, A.L. Lukes, J.E. Lang, K. Gaines, Quantitative crystallinity determinations for beta-lactam antibiotics by solution calorimetry: correlations with stability, J. Pharm. Sci. 67 (1978) 767–773.

[27] G. Buckton, P. Darcy, Int. J. Pharmaceut. 179 (1999) 141–158.

[28] C. Lavergne, C. Lacabanne, A review of thermally stimulated current, IEEE Electrical Insulation Magazine 9 (1993) 5–21.

[29] G.M. Venkatesh, M.E. Barnett, C. Owusu-Fordjour, M. Galop, H.L. Brown, A comparative evaluation of techniques for detecting low levels the amorphous phase in crystalline pharmaceutical materials, in: AAPS, San Francisco, 1998, Poster.

[30] G.P. Johari, M. Goldstein, Viscous liquids and the glass transition II secondary relaxation in glasses of rigid molecules, J. Chem. Phys. 53 (1970) 2372–2388.

[31] G.P. Johari, Effect of annealing on the secondary relaxations in glasses, J. Chem. Phys. 77 (1982) 4619–4626.

[32] N. Fagegaltier, A. Lamure, C. Lacabanne, A. Caron, H. Mifsud, M. Bauer, Thermal analysis of amorphous phase in a pharmaceutical drug, J. Thermal Anal. 48 (1997) 459–464.

[33] H. Tanaka, Two-order-parameter description of liquids. I. A general model of glass transition covering its strong and fragile limit (p. 3163–3174). II. Criteria for vitrification and predictions of our model (pp. 3175–3182). J. Chem. Phys. 111(1999) 3163–3174 and 3175–3182.

[34] D. Kivelson, J.-C. Pereda, K. Luu, M. Lee, H. Sakai, A. Ha, I. Cohen, G. Tarjus, Facts and speculation concerning low-temperature polymorphism in glass formers, in: J. Fourkas, D. Kivelson, U. Mohanty, K. Nelson (Eds.), ACS Symposium Series 676, Supercooled liquids: advances and novel applications, American Chemical Society, Washington, D.C, 1997.

[35] P.H. Poole, T. Grande, C.A. Angell, P.F. McMillan, Polymorphic phase transitions in liquids and glasses, Science 275 (1997) 322–323.

[36] E.F. Westrum, J.P. McCullough, Thermodynamics of crystals, in: D. Fox, M.M. Labes, A. Weissberger (Eds.), Physical Chemistry of the Organic Solid State, Interscience Publishers, New York, 1963.

[37] J.W. Christian, The Theory of Transformations in Metals and Alloys, Pergamon, Oxford, 1981.

[38] G. Adam, J.H. Gibbs, J. Chem. Phys. 43 (1965) 139–146.

[39] E. Fukuoka, M. Makita, Y. Nakamura, Glassy state of pharmaceuticals. V. Relaxation during cooling and heating of glass by differential scanning calorimetry, Chem. Pharm. Bull (39) (1991) 2087–2090, See also previous papers in this series.

[40] I.M. Hodge, Enthalpy Relaxation and Recovery in Amorphous Materials, J. Non-Crystalline Solids 169 (1994) 211–266.

[41] M. Reading, B.K. Hahn, B.S. Crowe, Method and apparatus for modulated differential analysis, 6 July 1993, US Patent 5 224 775, See also P. S. Gill, S. R. Sauerbrunn, M. Reading, J. Thermal Anal. 40 (1993) 931.

[42] V.L. Hill, Q.M.D. Craig, L.C. Feely, Characterisation of spray-dried lactose using modulated differential calorimetry, Int. J. Pharmaceut. 161 (1998) 95–107.

[43] C.A. Angell, J.M. Sare, E. Sare, J. Glass transition temperatures for simple molecular liquids and their binary solutions, J. Phys. Chem. 82 (1978) 2622.

[44] H.S. Kim, G.A. Jeffrey, Acta Cryst. B25 (1969) 2607.

[45] A. Streitwieser, C.H. Heathcock, Introduction to Organic Chemistry, Second Edition, Macmillan Publishing Co., Inc, New York, 1981.

[46] The Merck Index. 10th Edition. Merck & Co., Inc.: Rahway, NJ, 1983.

[47] E.A. Schmitt, D. Law, G.G.Z. Zhang, Nucleation and crystallization kinetics of hydrated amorphous lactose above the glass transition temperature, J. Pharm. Sci. 88 (1999) 291–296.

[48] T.C. Marwick, An X-ray study of mannitol, dulcitol and mannose, Nature 127 (1931) 11–12.

[49] G.W. McCrea, An X-ray study of mannitol, Nature 127 (1931) 162–163.

[50] A. Rye, H. Sorum, Crystalline modifications of d-mannitol, Acta Chem. Scand. 6 (1952) 1128–1129.

[51] H.M. Berman, G.A. Jeffrey, R.D. Rosentein, The crystal structures of the α' and β forms of d-mannitol, Acta Cryst. B24 (1968) 442–449.

[52] H.S. Kim, G.A. Jeffrey, R.D. Rosenstein, The crystal structure of the k form of d-mannitol, Acta Cryst. B24 (1968) 1449–1455.

[53] L. Walter-Levy, Sur les varietes crystallines du D-mannitol, C. R. Acad. Sci. Paris 267 (1968) 1779–1782.

[54] B. Debord, C. Lefebvre, A.M. Guyot-Hermann, J. Hubert, R. Bouche, J.C. Guyot, study of different crystalline forms of mannitol: comparative behaviour under compression, Drug Dev. Ind. Pharm. 13 (1987) 1533–1546.

[55] F.T. Jones, K.S. Lee, The optical and crystallographic

properties of three phases of mannitol, Microscope 18 (1970) 279–285.

[56] T.B. Grindley, M.S. McKinnon, R.E. Wasylishen, Towards understanding ^{13}C-N.M.R. chemical shifts of carbohydrates in the solid state. The spectra of D-mannitol polymorphs and of DL-mannitol, Carbohydr. Res. 197 (1990) 41–52.

[57] I. Pitkanen, P. Perkkalainen, H. Rautiainen, Thermoanalytical studies of phases of D-mannitol, Thermochim. Acta 214 (1993) 157–162.

[58] A. Burger, J.-O. Henck, S. Hetz, J.M. Rollinger, A.A. Weissnicht, Energy/temperature diagram and compression behavior of the polymorphs of mannitol, J. Pharm. Sci. 89 (2000) 457–468.

[59] L. Yu, N. Milton, E.D. Groleau, D.S. Mishra, R.E. Vansickle, Existence of a mannitol hydrate during freeze-drying and practical implications, J. Pharm. Sci. 88 (1999) 196–198.

[60] J.W. Du Ross, Modification of the crystalline structure of sorbitol and its effect on tableting characteristics, Pharmaceutical Technology, September(1984), 42–53.

[61] M. Siniti, J. Carre, J.P. Bastide, J.M. Letoffe, P. Claudy, The thermal behavior of hexitols. part 2. polymorphism of dulcitol, Thermochim. Acta 224 (1993) 105–110.

[62] F. Sussich, R. Urbani, F. Princivalle, A. Cesaro, Polymorphic amorphous and crystalline forms of trehalose, J. Am. Chem. Soc. 120 (1998) 7893–7899.

[63] A.I. Kim, M.J. Akers, S.L. Nail, The physical state of mannitol after freeze-drying: effects of mannitol concentration, freezing rate, and a noncrystallizing cosolute, J. Pharm. Sci. 87 (1998) 931–935.

[64] R.K. Cavatur, R. Suryanarayanan, Characterization of frozen aqueous solutions by low temperature X-ray powder diffractometry, Pharm. Res. 15 (1998) 194–199.

[65] T. Kovalcik, J.K. Guillory, The stability of cyclophosphamide in lyophilized cakes. part I. mannitol, lactose, and sodium bicarbonate as excipients, J. Parenteral Sci. Tech. 42 (1988) 29–39.

[66] K. Izutsu, S. Yoshioka, T. Terao, Effect of mannitol crystallinity on the stabilization of enzymes during freeze-drying, Chem. Pharm. Bull. 42 (1994) 5–8.

[67] J. Jolley, Photogr. Sci. Eng. 14 (1970) 167–177.

[68] V. Andronis, G. Zografi, Crystal nucleation and growth of indomethacin polymorphs from the amorphous state, J. Non-Crystalline Solids 271 (2000) 236–248.

[69] N.A. Willaims, Y. Lee, G.P. Polli, T.A. Jennings, The effect of cooling rate on solid phase transitions and associated vial breakage occurring in frozen mannitol solutions, J. Parenteral Sci. Tech. 40 (1986) 135–141.

[70] N.A. Williams, T. Dean, Vial breakage by frozen mannitol solutions: correlation with thermal characteristics and effect of stereoisomerism, additives and vial configuration, J. Parenteral Sci. Tech. 45 (1991) 94–100.

[71] K.F. Kelton, Nucleation in silicate and metallic glasses, Int. J. Non-Equilibrium Proc. 11 (1998) 141–168.

[72] H. Senapati, R.K. Kadiyala, C.A. Angell, Single- and two-step calorimetric studies of crystallization kinetics in simple ionic glass-forming liquids. 1. Ca(NO3)2-KNO3 system, J. Phys. Chem. 95 (1991) 7050–7054.

[73] V. Andronis, G. Zografi, Molecular mobility of supercooled amorphous indomethacin determined by dynamic mechanical analysis, Pharm. Res. 14 (1997) 410–414.

[74] N.T. Correia, C. Alvarez, J.J.M. Ramos, M. Descamps, Molecular motions in molecular glasses as studied by thermally stimulated depolarization currents (TSDC), Chem. Phys. 252 (2000) 151–163.

[75] F. Qi, K.U. Schug, S. Dupont, A. Döß, R. Böhmer, H. Sillescu, H. Kofshorn, H. Zimmerman, Structural relaxation of the fragile glass-former propylene carbonate studied by nuclear magnetic resonance, J. Chem. Phys. 112 (2000) 9455–9462.

[76] C.T. Moynihan, A.J. Easteal, J. Wilder, Dependence of the glass transition temperature on heating and cooling rate, J. Phys. Chem. 78 (1974) 2673.

[77] C.T. Moynihan, S.-K. Lee, M. Tatsumisago, T. Minami, Estimation of activation energies for structural relaxation and viscous flow from dta and dsc experiments, Thermochim. Acta 280–281 (1996) 153–162.

[78] P. Skoglund, A. Fransson, Thermochim. Acta 276 (1996) 27–39.

[79] G.W.H. Hohne et al., Thermochim. Acta 221 (1993) 129–137.

[80] C.T. Moynihan, correlation between the width of the glass transition region and the temperature dependence of the viscosity of high-tg glasses, J. Am. Ceram. Soc. 76 (1993) 1081.

[81] R. Bruning, M. Sutton, Fragility of glass-forming systems and the width of the glass transition, J. Non-Crystalline Solids 205–207 (1996) 480–484.

[82] O. Bustin, M. Descamps, Slow structural relaxations of glass-forming maltitol by modulated DSC calorimetry, J. Chem. Phys. 110 (1999) 10982–10992.

[83] P.M. Mehl, Calorimetric investigation of the glass transition and relaxation in 60:40 ethylene glycol:water. Part 1. Isothermal annealing experiments, Thermochim. Acta 213 (1993) 177–197.

[84] P.M. Mehl, Determination of enthalpy relaxation times using traditional differential scanning calorimetry for glycerol and for propylene glycol, Thermochim. Acta 272 (1996) 201–209.

[85] S.L. Shamblin, G. Zografi, Enthalpy relaxation in binary amorphous mixtures containing sucrose, Pharm. Res. 15 (1998) 1828–1834.

[86] B.C. Hancock, S.L. Shamblin, G. Zografi, Molecular mobility of amorphous pharmaceutical solids below their glass transition temperatures, Pharm. Res. 12 (1995) 799–806.

[87] C.A. Angell, Relaxation in liquids, polymers and plastic crystals -strong/fragile patterns and problems, J. Non-Crystalline Solids. 131–133 (1991) 13.

[88] R. Bohmer, K.L. Ngai, C.A. Angell, D.J. Plazek, Nonexponential relaxations in strong and fragile glass formers, J. Chem. Phys. 99 (1993) 4201–4209.

[89] J.L. Green, J. Fan, C.A. Angell, The protein-glass analogy: some insights from homopeptide comparisons, J. Phys. Chem. 98 (1994) 13780–13790.

[90] V. Samouillan, A. Lamure, C. Lacabanne, Dielectric relaxa-

tion of collagen and elastin in the dehydrated state, Chem. Phys. 255 (2000) 259–271.

[91] I.M. Hodge, J. Non-Crystalline Solids 202 (1996) 164–172.

[92] R. Richert, C.A. Angell, J. Chem. Phys. 108 (1998) 9016.

[93] J.L. Green, K. Ito, K. Xu, C.A. Angell, Fragility in liquids and polymers: new, simple quantitations and interpretations, J. Phys. Chem. B 103 (1999) 3991–3996.

[94] K. Ito, C.T. Moynihan, C.A. Angell, Thermodynamic determination of fragility in liquids and a fragile-to-strong liquid transition in water, Nature 398 (1999) 492–495.

[95] B.C. Hancock, C.R. Dalton, M.J. Pikal, S.L. Shamblin, A pragmatic test of a simple calorimetric method for determining the fragility of some amorphous pharmaceutical materials, Pharm. Res. 15 (1998) 762–767.

[96] N.B. Olsen, Scaling of beta-relaxation in the equilibrium liquid state of sorbitol, J. Non-Crystalline Solids 235–237 (1998) 399–405.

[97] H. Wagner, R. Richert, Spatial Uniformity of the beta-Relaxation in D-sorbitol, J. Non-Crystalline Sol. 242 (1998) 19–24.

[98] T. Hikima, M. Hanaya, M. Oguni, Microscopic observation of a peculiar crystallization in the glass transition region and beta-process as potentially controlling the growth rate in triphenylethylene, J. Mol. Structure 479 (1999) 245–250.

[99] T.G. Fox, Influence of diluent and of copolymer composition on the glass transition temperature of a polymer system, Bull. Am. Phys. Soc. Ser. II 1 (1956) 123.

[100] P.R. Couchman, The composition-dependent glass transition: relations between temperature, pressure, and composition, Polymer Eng. and Sci. 24 (1984) 135.

[101] M. Gordon, J.S. Taylor, Ideal copolymers and the second-order transitions of synthetic rubbers. i. non-crystalline copolymers, J. Appl. Chem. 2 (1952) 493.

[102] B.C. Hancock, G. Zografi, The relationship between the glass transition temperature and the water content of amorphous pharmaceutical solids, Pharm. Res. 11 (1994) 471–477.

[103] T. Matsumoto, G. Zografi, Physical properties of solid molecular dispersions of indomethacin with poly(vinylpyrrolidone) and poly(vinylpyrrolidone-co-vinylacetate) in rela-

tion to indomethacin crystallization, Pharm. Res. 11 (1999) 1722–1728.

[104] E.Y. Shalaev, G. Zografi, How does residual water affect the solid-state degradation of drugs in the amorphous state, J. Pharm. Sci. 85 (1996) 1137–1141.

[105] M.C. Lai, E.M. Topp, Solid-state chemical stability of proteins and peptides, J. Pharm. Sci. 88 (1999) 489–500.

[106] W.Q. Sun, P. Davidson, H.S.O. Chan, Protein stability in the amorphous carbohydrate matrix: relavance to anhydrobiosis, Biochim. Biophys. Acta 1425 (1998) 245–254.

[107] R.H.M. Hatley, J.A. Blair, Stabilization and delivery of labile materials by amorphous carbohydrates and their derivatives, J. Mol. Catalysis B: Enzymatic 7 (1999) 11–19.

[108] J. Bernstein, Conformational polymorphism, in: G.R. Desiraju (Ed.), Organic Solid State Chemistry, Elsevier, Amsterdam, 1987.

[109] E.Y. Shalaev, Q. Lu, M. Shalaeva, G. Zografi Acid-catalyzed inversion of sucrose in the amorphous state at very low levels of residual water, Pharm. Res. 17 (2000) 366–370.

[110] M. Yoshioka, B.C. Hancock, G. Zografi, Crystallization of indomethacin from the amorphous state below and above its glass transition temperature, J. Pharm. Sci. 83 (1994) 1700–1705.

[111] R.H.M. Hatley, Glass fragility and the stability of pharmaceutical preparations - excipients selection, Pharm. Dev. Technol. 2 (1997) 257–264.

[112] L.M. Crowe, D.S. Reid, J.H. Crowe, Is trehalose special for preserving dry biomolecules?, Biophys. J. 71 (1996) 2087–2093.

[113] S. Magazu, G. Maisano, P. Migliardo, E. Tettamanti, V. Villari, Transport phenomena and anomalous glass-forming behavior in alpha, alpha-trehalose aqueous solutions, Mol. Phys. 96 (1999) 381–387.

[114] C. Branca, S. Magazu, G. Maisano, P. Migliardo, Anomalous cryoprotective effectiveness of trehalose: Raman scattering evidence, J. Chem. Phys. 111 (1999) 281–287.

Exhibit D

REVIEW ARTICLE

Characteristics and Significance of the Amorphous State in Pharmaceutical Systems

BRUNO C. HANCOCK[*x] AND GEORGE ZOGRAFI[†]

Abstract ☐ The amorphous state is critical in determining the solid-state physical and chemical properties of many pharmaceutical dosage forms. This review describes the characteristics of the amorphous state and some of the most common methods that can be used to measure them. Examples of pharmaceutical situations where the presence of the amorphous state plays an important role are presented. The application of our current knowledge to pharmaceutical formulation problems is illustrated, and some strategies for working with amorphous character in pharmaceutical systems are provided.

Figure 1—Schematic diagram of the most common ways in which amorphous character is induced in a pharmaceutical system.

Introduction

During the final stage of developing a synthetic procedure for a new drug entity, a great deal of emphasis is placed on obtaining material of high purity, and reproducibility in terms of its physical, chemical, and biological properties. Every effort is made to ensure a high degree of crystallinity, wherein the molecules have regular and well-defined molecular packing, and emphasis is also placed on whether or not the compound can exist in polymorphic or solvated crystal forms.[1] These forms can have different thermodynamic properties (e.g., melting temperature, vapor pressure, solubility), and a knowledge of their existence is required to anticipate spontaneous changes in the properties of the solid during storage and/or handling of the material. It is also possible that upon isolation the material will be obtained in a fully or partially amorphous state.[2] The four most common means by which amorphous character is induced in a solid are shown in Figure 1. These are condensation from the vapor state, supercooling of the melt, mechanical activation of a crystalline mass (e.g., during milling), and rapid precipitation from solution (e.g., during freeze-drying or spray drying). Amorphous character is common with polymeric molecules used as excipients, and

large peptides and proteins used as therapeutic agents, and it can also occur with small organic and inorganic molecules. When a system consists of multiple components, as with pharmaceutical formulations, it is possible that amorphous solid-state solutions can form analogous to liquid solutions. Water vapor can also be absorbed by an amorphous solid to form an amorphous solid solution.

The three-dimensional long-range order that normally exists in a crystalline material does not exist in the amorphous state, and the position of molecules relative to one another is more random as in the liquid state. Typically amorphous solids exhibit short-range order over a few molecular dimensions and have physical properties quite different from those of their corresponding crystalline states. In Figure 2 we schematically plot the enthalpy (H) or specific volume (V) of a solid substance as a function of its temperature. For a crystalline material at very low temperatures we see a small increase in enthalpy and volume with respect to temperature, indicative of a certain heat capacity (C_p) and thermal expansion coefficient (α). There is a discontinuity in both H and V at the melting temperature (T_m) representing the first-order phase transition to the liquid state. Upon rapid cooling of the melt the values of H and V may follow the equilibrium line for the liquid beyond the melting temperature into a

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Figure 2—Schematic depiction of the variation of enthalpy (or volume) with temperature.

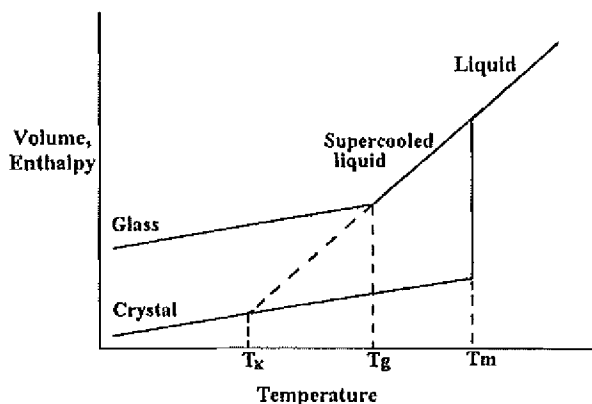"supercooled liquid" region. On cooling further a change in slope is usually seen at a characteristic temperature known as the glass transition temperature (T_g). At T_g the properties of the glassy material deviate from those of the equilibrium supercooled liquid to give a nonequilibrium state having even higher H and V than the supercooled liquid. As a result of its higher internal energy (e.g., $\approx$25 kJ·mol^{-1} for cephalosporins[3]) the amorphous state should have enhanced thermodynamic properties relative to the crystalline state (e.g., solubility,[4] vapor pressure) and greater molecular motion. We would also expect amorphous systems to exhibit greater chemical reactivity[3] and to show some tendency to spontaneously crystallize, possibly at different rates below and above T_g.[5] From a pharmaceutical perspective we have an interesting situation. The high internal energy and specific volume of the amorphous state relative to the crystalline state can lead to enhanced dissolution and bioavailability,[4] but can also create the possibility that during processing or storage the amorphous state may spontaneously convert back to the crystalline state.[5]

In considering the importance of the amorphous state in pharmaceutical systems we must direct our attention to two main situations. In the first, a material may exist intrinsically in the amorphous state or it may be purposefully rendered amorphous and we would like to take advantage of its unique physical chemical properties. Under these circumstances we usually want to develop strategies to prevent physical and chemical instability of the amorphous sample. In the second case, we may be dealing with a crystalline material that has been inadvertently rendered amorphous during processing. This type of amorphous character usually exists predominately at surfaces at levels not easily detected and has the potential to produce unwanted changes in the physical and chemical properties of the system. In this situation we usually want to process the system so that the amorphous portions of the solid are converted back to the most thermodynamically stable crystalline state.

Definition and Description of the Amorphous State

The rapid cooling of a liquid below its melting point (T_m) may lead to an amorphous state with the structural characteristics of a liquid, but with a much greater viscosity (Figures 2 & 3). The enthalpy and volume changes immediately below T_m exhibit no discontinuity with those observed above T_m, so we consider this amorphous state to be an equilibrium "supercooled" liquid. This amorphous state is also called the "rubbery state" because of the macroscopic properties of amorphous solids in this region. We can further characterize

this state by considering its rate and extent of molecular motions. The average time scale of molecular motions within a supercooled liquid is usually less than 100 s, the viscosity is typically between 10^{-3} and 10^{12} Pa·s (Figure 3), and both properties are strongly temperature dependent.[6–10] Cooling the supercooled liquid even further appears to reduce the molecular mobility of the material to a point at which the material is kinetically unable to attain equilibrium in the time scale of the measurement as it loses its thermal energy, resulting in a change in the temperature dependence of the enthalpy and volume. The temperature at which this occurs is the experimentally observed glass transition temperature (T_g). Below T_g the material is "kinetically frozen" into a thermodynamically unstable glassy state with respect to both the equilibrium liquid and the crystalline phase, and any further reduction in temperature has only a small effect upon its structure. Molecular motions in glasses typically occur over a period in excess of 100 s, and viscosities are usually greater than 10^{12} Pa·s.[6–10] Many of the physical properties of glassy amorphous materials (e.g., thermal expansion coefficient) are different from those of the corresponding supercooled liquid above T_g.

The molecular processes which contribute to the glass transition are currently the subject of intensive research and debate. Whether the changes in thermodynamic properties (e.g., specific heat, volume) that are seen during cooling (or reheating) are due to a real thermodynamic phase transition or are of purely kinetic origin is a controversial issue, and no theory has yet been proposed which accounts for all the observed experimental features. Several excellent reviews which describe the current thinking in this field have been published.[6–8,10,11] Models based on statistical mechanical or free volume theories are the simplest and most widely invoked. Polymer scientists, metallurgists, ceramists, etc. each have their preferred approaches with specific advantages for the materials and processes with which they are working. From Figure 2 it can be seen that the glass transition can be considered to be a thermodynamic requirement for a supercooled liquid since without such a transition the amorphous material would attain a lower enthalpy than the crystalline state at some critical temperature and would eventually attain a negative enthalpy. This critical temperature is known as the Kauzmann temperature (T_K) and is thought to mark the lower limit of the experimental glass transition (T_g) and to be the point at which the configurational entropy of the system reaches zero.[9,10] Experimental studies of the glass transition are complicated by the existence of many different modes of molecular motion in most systems (e.g., rotational or translational), changes in the scale and type of motions with temperature, and cooperativity or coupling of molecular motions. One can only say for certain that at T_g the mean molecular relaxation time (τ) associated with the predominant molecular motions is about 100 s and that T_g can be expected to vary with experimental heating and cooling rates, sample molecular mass,[12,13] sample history, sample geometry,[14,15] and sample purity.[16] The experimental glass transition temperature is also influenced by the choice of technique used to measure it because of the varying sensitivities of available techniques to different types and speeds of molecular motions.

The temperature dependence of molecular motions directly determines many important physical properties of amorphous materials, including the location of the glass transition temperature and the ease of glass formation. This temperature dependence is most frequently described using the empirical Vogel-Tammann-Fulcher (VTF) equation:[7,8,10]

$$\tau = \tau_0 \exp(DT_0/T - T_0) \qquad (1)$$

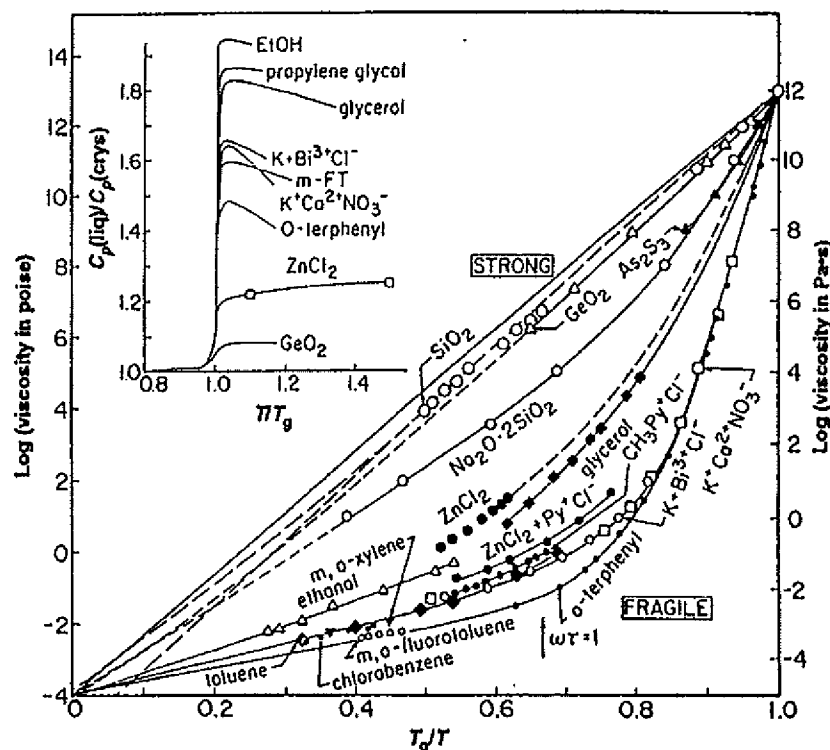where τ is the mean molecular relaxation time, T is the

Figure 3—Molecular mobility (or viscosity) of amorphous materials as a function of normalized temperature above T_g.[7,8] Reprinted with permission from ref 8. Copyright 1995 American Association for the Advancement of Science.

temperature, and τ_0, D, and T_0 are constants. The value of T_0 in the VTF equation is believed to correspond to the theoretical Kauzmann temperature (T_K), and τ_0 can be related to the relaxation time constant of the unrestricted material.[7,8] When T_0 is 0, the familiar Arrhenius equation is obtained, and D is directly proportional to the activation energy for molecular motion. When T_0 is greater than 0, there is a temperature dependent apparent activation energy. The Williams—Landel—Ferry (WLF) equation[17] describing the temperature dependence of viscosity (η) in polymers above T_g is a special case of the VTF equation:

$$\eta = \eta_g \exp\{C_1(T - T_g)/(C_2 + (T - T_g))\} \qquad (2)$$

where η_g is the mean viscosity at T_g and C_1 and C_2 are constants. This equation can be derived from first principles based on polymer free volume theories. The constants C_1 and C_2 are found to be quite universal for a range of polymers[17] and are equivalent to $DT_0/(T_g - T_0)$ and $(T_g - T_0)$, respectively, in the VTF equation. The WLF equation has been shown to fit viscosity data for several small organic molecules using the universal constants,[18–20] making it useful for predicting the relaxation behavior or molecular mobility of amorphous pharmaceutical solids. However, it is important to recognize that this is not always the case and such predictions cannot always be assumed to be accurate.

Depending upon the magnitude and temperature dependence of the apparent activation energy for molecular motions near and above T_g in supercooled liquids, it is possible to classify them as either "strong" or "fragile" amorphous systems (Figure 3).[7,8] A strong liquid typically exhibits Arrhenius-like changes in its molecular mobility with temperature and a relatively small change in heat capacity at T_g. Proteins are good examples of strong glass formers, with their changes in heat capacity at T_g often being so small that they cannot be detected using standard calorimetry techniques.[21] A fragile

supercooled liquid has a much stronger temperature dependence of molecular mobility near T_g and a relatively large change in heat capacity at T_g and will typically consist of nondirectionally, noncovalently bonded molecules (e.g., ethanol). The constant D in the VTF equation is an indicator of fragility, with low values (<10) corresponding to very fragile glass formers and high values (>100) indicating strong glass-forming tendencies. The value of T_0 in the VTF equation is also linked to the fragility of the system with $(T_g - T_0) > 50$ typical of strong glass formers and $(T_g - T_0) < 50$ usual for fragile materials. A simple graphical means of ranking materials in terms of their strength/fragility is to plot the molecular mobility (or viscosity) as a function of the temperature normalized to the experimental glass transition temperature (e.g., Figure 3).[7,8] A "rule of thumb" for determining fragility without relaxation time data has also been proposed based on the relative magnitudes of the melting and glass transition temperatures: strong, T_m/T_g (in K) >1.5; fragile, T_m/T_g (in K) < 1.5)[7,8] (Table 1). (See Note Added in Proof.)

The extent of departure of a *glass's* properties from equilibrium is determined by its formation conditions, so we can presume the existence of multiple metastable glasses *below* T_g (Figure 2),[2,3] and even polyamorphic glasses that convert via first-order transitions.[22–24] As a result of this, the temperature dependence of molecular motions *below* the glass transition temperature is highly dependent upon the conditions under which the glass was formed.[12] This temperature dependence is generally less extreme than above T_g and more linear, with some authors proposing an Arrhenius-like relationship. That molecular motions do occur below T_g is unquestionable, and the consequences of the relaxation or "aging" of glassy materials have been widely reported. For example, Guo *et al.*[25] described effects upon the film-coat water permeability and dissolution rate of film coated tablets, and Byron and Dalby[26] studied the effects of aging on the permeability of poly(vinyl alcohol) films to a model water soluble

Table 1—Measured Physical Properties of Some Amorphous Pharmaceutical Materials[a]

Material	M_w	T_m (K)	T_g (K)	T_m/T_g	ΔC_p (J·g^{-1}·K^{-1})	$\rho_{crystal}$ (kg·m^{-3})	ρ_{amorph} (kg·m^{-3})
Indomethacin	358	438	320	1.37	0.466	1.38	1.32
Sucrose	342	453	348	1.30	0.544	1.59	1.43
Lactose (anhydrous)	342	486	383	1.27	0.472	1.60	1.48
Trehalose (anhydrous)	342	476	385	1.24	0.534	1.58	1.49
Dextran	$\approx 5 \times 10^5$	—	498	—	0.400	—	0.92
Poly(vinylpyrrolidone)	$\approx 1 \times 10^6$	—	458	—	0.260	—	1.25
Water[b]	18	273	136	2.01	0.100	<0.95	≈ 0.95

[a] M_w = molecular mass; T_g = glass transition temperature; T_m = melting temperature; ΔC_p = heat capacity change at T_g; ρ = density. [b] Reference 134.

drug. The effects of aging are often detrimental, but they can also be used to improve a product's performance with a deliberate "annealing" process. This strategy is particularly useful when small amounts of amorphous character have been unintentionally introduced into a system by high-energy processing (see later).[27,28] The time scale of molecular motions in a glass is much longer than above T_g ($\tau \gg 100$ s) and requires different experimental techniques for its study. In almost all cases the molecular relaxation processes that occur in glasses follow a nonexponential function. This nonexponentiality has been widely studied and modeled[29] and appears to be the result of a heterogeneous microstructure within glasses which leads to a distribution of types and rates of molecular motion under any given time and temperature conditions. The reader is referred to some excellent reviews for detailed information on the application of these models to glassy systems.[12,29] The empirical Kohlrausch—Williams—Watts (KWW) stretched exponential function is most often used to describe the distribution of molecular motions:[10]

$$\phi(t) = \exp\{-(t/\tau)^\beta\} \qquad (3)$$

where $\phi(t)$ is the extent of relaxation at time t, τ is the mean molecular relaxation time, and β is a constant. A β value of unity corresponds to a single relaxation time with exponential behavior. The smaller the value of β, the more the distribution of molecular motions deviates from a single exponential. β has been shown to correspond to the strength/fragility of the material above T_g, but no similar relationship has yet been established below T_g. By fitting data to the KWW function it is possible to determine the mean molecular relaxation time (τ) and β for any well-defined glass.[30] A general means of ranking glasses in terms of the temperature dependence of molecular motions, similar to Angell's strong/fragile classification system above T_g, would be of great use to pharmaceutical materials scientists but has not yet been developed because of the greater complexities of the glassy state.

Perhaps the most important question relating to amorphous pharmaceutical systems is, *At what temperature do the molecular motions responsible for physical and chemical instabilities cease to become likely over the lifetime of that particular system?*[30] It has been suggested that this lower temperature limit might correspond to the Kauzmann temperature (T_K). Although this appears to be the case for some systems, there also appears to be an influence from the strength/fragility of the system, and also from whether or not the molecular motions that are responsible for the glass transition and any instabilities are identical.[30] Mean molecular relaxation times have been reported for several pharmaceutical glass formers as a function of temperature following enthalpy relaxation and thermomechanical relaxation experiments, and the temperature of negligible molecular mobility during a 3-year shelf life varied according to (i) the method used to assess the molecular motions and (ii) the identity of the glass former.[30] As yet there is no reliable means of predicting the temperature of negligible molecular mobility in amorphous solids, and thus a conservative approach is

required when defining storage and processing conditions for amorphous pharmaceutical systems (see later).

The behavior of amorphous systems as defined in Figure 2 is dependent upon the assumption of constant pressure and composition. Pressure effects upon amorphous materials have not been widely studied but are likely to be significant with effects on molecular packing potentially modifying the glass transition temperature, the thermal expansion behavior, and the strength/fragility of a supercooled liquid.[10,31,32] From a practical perspective the glass transition temperature of a system containing volatile components may only be experimentally accessible at elevated pressures. For example, the widespread and significant plasticizing effects of sorbed water vapor in high-T_g amorphous polymers have only recently been fully realized because of advances in sample-handling methods which allow samples of varying water content to be sealed at ambient temperature and then heated through T_g without loss of their sorbed water vapor.[33] The properties of a glassy amorphous solution prepared by lyophilization are also likely to be significantly different from those of the same system prepared at ambient pressure since the reduced pressure within a lyophilization chamber will affect the structure of the amorphous cake that is formed and also the composition of the solution through the primary and secondary drying processes. Angell *et al.*[34] have noted that for aqueous solutions the fragility of the supercooled solution is dependent upon the solute concentration in the solution. From the limited data available it can be concluded that some supercooled aqueous solutions become stronger as they become more dilute (*e.g.*, sugars), whereas others become more fragile (*e.g.*, electrolytes, salts). The type of behavior observed appears to be linked to the extent of hydrogen bonding in the aqueous solution. The fragility of such mixed systems may also be related to the ideality of their mixing behavior. Simple mixing rules have been used by many authors[35,36] to describe the variation of the glass transition temperature with blend composition; however, the effects of nonidealities (*e.g.*, immiscibility, molecular size differences, specific interactions, *etc.*) are often significant. The simplest and most reliable approach for use with amorphous pharmaceutical materials appears to be a modified Gordon—Taylor equation[35,36] which is based on free volume theories with some simplifying assumptions. For simple two-component mixtures,

$$T_{gmix} = (w_1 T_{g1} + K w_2 T_{g2})/(w_1 + K w_2) \qquad (4)$$

where T_g is the glass transition temperature, w_1 and w_2 are the weight fractions of components 1 and 2, and K can be calculated from the densities (ρ) and glass transition temperatures (T_g) of the components:

$$K = (T_{g1}\rho_1)/(T_{g2}\rho_2) \qquad (5)$$

Similar equations can be readily derived for mixtures of more than two components. A perfecty miscible system will display a single sharp glass transition event. Immiscibility, incompatibility, or nonideality is often indicated by a poor fit to the

theoretical equation, the appearance of more than one T_g, or "broadening" of the glass transition event. Deviations from ideal behavior can also be identified and their most likely causes assessed using the graphical approach of Schneider and co-workers.[36,37] Deviations usually occur over discrete composition ranges and often can be explained in terms of molecular size effects and the disappearing free volume of the high-T_g component at lower temperatures and compositions.[38,39] Such an approach is analogous to percolation theories and has considerable potential for describing mixed amorphous systems. Simple solution theories also can be used to describe such systems and to provide a qualitative understanding of the important factors regulating the glass transition in pharmaceutical systems. For example, it is likely that, when a macromolecule is mixed in small amounts with an amorphous small molecule, it will introduce a considerable excess free volume to the system because of its much larger molecular size. In this situation the glass transition temperature of the mixture probably will not be elevated as much as predicted by theory. The addition of low levels of a small molecule to an amorphous macromolecular system probably will be much less disruptive. Both materials will make near ideal contributions to the overall free volume of the mixture, and in this instance the predictions of the mixing equations are likely to be quite accurate for at least the first 50 K change in T_g. This is very important since the presence of very low levels of low molecular weight contaminants or additives (including water vapor) is predicted and observed to have significant plasticizing effects on pharmaceutical glasses,[36] whereas the addition of low levels of high molecular mass additives often has minimal antiplasticizing effect.[39] It should be noted that the concept of a critical additive composition (W_g) at which a glassy macromolecular material is sufficiently plasticized by a low molecular weight penetrant that it transforms to a rubbery amorphous solid under ambient conditions has been described by several authors.[33,40]

Pharmaceutical solids rarely exist as 100% crystalline or 100% amorphous phases so it is necessary at this point to consider how partially crystalline or amorphous systems are likely to behave. The coexistence of two thermodynamically different states of a material will probably result in (i) significant and measurable structural heterogeneities and (ii) batch to batch variations in physical properties. The presence of one phase in another can act as a focal point for spontaneous phase transitions such as crystallization.[28,41,42] In addition, as each phase is intimately dispersed in the other, there may not be complete independence of their behavior. For example, the dispersion of crystalline drug in an amorphous carrier has been reported to alter the observed glass transition temperature of the amorphous phase.[43] For macromolecules there may even be molecules which are part of both crystalline and amorphous domains physically linking the two regions together. Partially ordered systems have traditionally been described using either "one-state" or "two-state" models.[4,28,41,42] In the two-state model, domains of material are assumed to be either 100% amorphous or 100% crystalline and they coexist side by side in a molecular mixture. This type of system can be simulated to some extent by making physical mixtures of reference samples of crystalline and amorphous materials.[3] The one-state model consists of domains which are truly partially crystalline and in which the molecules have formed a semiordered structure as a result of being restricted in their motion during crystallization, or following the disruption of a more perfect crystalline state. The one-state model seems intuitively more likely than the two-state model but raises many questions which cannot be readily answered by studying mixtures of the reference crystalline and amorphous materials. In metallic systems there is also a state known as the "nanocrystalline phase" which has properties intermediate
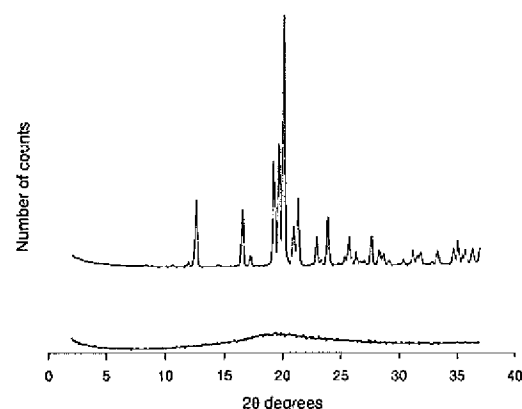

Figure 4—X-ray powder diffraction patterns for amorphous (bottom) and crystalline (top) lactose.

to those of the amorphous and crystalline states,[44] and the concept of "glassy" or "plastic" crystals has recently been described.[45] Clearly the ability to distinguish between crystalline and amorphous states of a material and to be able to quantify one phase in the presence of the other is critical to the successful design and production of amorphous pharmaceutical systems.

Characterization of the Amorphous State

Upon passing into the supercooled liquid state or through the glass to rubber transition it is possible to observe changes in a multitude of material physical properties including density, viscosity, heat capacity, X-ray diffraction, and diffusion behavior. Techniques which measure these properties (directly or indirectly) can be used to detect the presence of an amorphous material (glass or rubber), and some of these methods are sensitive enough to allow quantification of the amount of molecular order or disorder (amorphous content) in a partially crystalline system.

As there is no long-range three-dimensional molecular order associated with the amorphous state, the diffraction of electromagnetic radiation (e.g., X-rays) is irregular compared to that in the crystalline state (Figure 4). Diffraction techniques are perhaps the most definitive method of detecting and quantifying molecular order in any system, and conventional, wide-angle and small-angle diffraction techniques have all been used to study order in systems of pharmaceutical relevance.[3,5,41] The specificity and accurate quantitative nature of these nondestructive techniques make them first line choices for studying partially crystalline pharmaceutical materials. Conventional X-ray powder diffraction measurements can be used to quantify non-crystalline material down to levels of about 5%[41] and with temperature and environmental control can also be used to follow the kinetics of phase transformations, or to quantify the presence of a crystalline drug in an amorphous excipient matrix.[46] Small-angle X-ray measurements have been used to study subtle structural (density) changes in polymers in the glassy state upon annealing,[47] and neutron scattering is gaining wider use in the characterization of short-range two-dimensional order in amorphous materials.[48] It should be remembered that diffraction techniques only "see" molecular order, and thus disorder is only implied.

The irregular arrangement of molecules in the amorphous state usually causes them to be spaced further than in a crystal so that the specific volume is greater and the density lower than that of the crystal, and we say that there is a greater "free volume" (Figure 2). Highly accurate measure-

ments of volume or density are difficult, and the magnitudes of differences involved are fairly small (Table 1), so such determinations are not a method of choice for characterizing amorphous pharmaceutical systems. The use of gas displacement pycnometry for quantifying the amorphous content of partially crystalline pharmaceutical systems has been described by Saleki-Gerhardt et al.,[41] and the accuracy achieved was about ±10%. Liquid displacement pycnometry has also been used to determine the crystallinity of several starch samples.[49] This approach has proved useful for quantifying low levels of disorder in crystalline pharmaceutical samples.[50] Precise dilatometric techniques are widely used for the study of amorphous polymers,[51] and these techniques could be useful for pharmaceutical systems; however, the methods are time-consuming and quite difficult to perform and so would not be suitable for routine use.

The most characteristic property of the amorphous state is its viscosity (approximately $<10^{12}$ Pa·s above T_g and $>10^{12}$ Pa·s below T_g). The greater free volume and molecular disorder of an amorphous material compared to its crystalline counterpart result in the mechanical properties of amorphous systems (viscosity, elastic modulus) being much more like those of a liquid than those of a solid. In some supercooled liquids a breakdown of the Stokes–Einstein relationship between the viscosity and the rate of diffusion (or molecular mobility) has been observed.[52] It is thought that the breakdown of this fundamental relationship may be due to the decoupling of certain modes of molecular motion in the amorphous state.[52,53] Methods which are used to measure the viscosity of amorphous materials are quite specialized and include the bending of rods or curved fibers below T_g[54] and the torsion pendulum and falling-sphere methods above T_g.[19] Experimental difficulties are often quite significant, and thus such measurements are usually feasible only in a specially equipped research laboratory.

Diffusion-controlled processes (such as gas transport, self-diffusion, crystallization, and some chemical reactions) which are all closely linked to the viscosity of the amorphous matrix have been the subject of many studies of glassy and rubbery amorphous materials.[23] As a consequence of the greater free volume in amorphous materials, diffusive transport processes are usually significantly more rapid and isotropic than in crystals. Often different diffusion rates are observed above and below T_g, with significantly faster diffusion occurring above T_g. This phenomenon can be used to identify the glass transition temperature in some amorphous systems. The sensitivity of transport processes to small fractions of crystalline order has yet to be clearly defined, although it appears that ordered domains can slow down the rate of diffusion. There is an intuitive connection between the viscosity of an amorphous system and the rate of diffusion within it. The well-known theoretical models developed for the liquid state are not directly applicable to amorphous systems, but models specifically derived for amorphous systems are numerous, with the most popular being based on thermodynamic and polymer free volume theories.[55,56] It is very difficult to make general predictions about diffusion behavior in amorphous systems because of the large number of system specific variables which can have an effect upon transport properties. It is possible to rank the rates of diffusion in the order liquid > rubber > glass > crystal, assuming all other factors to be approximately equal. Transport properties of amorphous pharmaceutical materials are important because they can be exploited to control drug release in modified release dosage forms (e.g., transdermal patches). The methods used to study transport phenomena in amorphous pharmaceutical systems include diffusion cells, NMR measurements,[57] and molecular dynamics simulations.[58]

The use of molecular probes (e.g., fluorescent or phospho-

rescent) to determine the physical properties of amorphous materials is becoming quite common.[59,60,61] These techniques rely upon the regulation of a probe's mobility by the amorphous system under consideration. As with all probe-based techniques, the accuracy of the data obtained relies on the choice of a probe which does not alter the properties of the amorphous material while at the same time being sensitive to those same properties. Water, for example, will plasticize most amorphous materials, and its own mobility is not necessarily affected by the state of its amorphous surroundings,[57] so it usually makes a very poor probe for amorphous systems. Phosphorescent probe studies at different temperatures with amorphous sucrose have shown changes in probe mobility around the calorimetric glass transition of the sugar,[59] and similar findings have been reported with sucrose–water, glycerol–water, and malto-oligosaccharide–water glasses using spin-probe electron spin resonance spectroscopy.[59,60,61] Quantification of the percent order and disorder is not currently possible using probe techniques, although probe mobility is likely to be affected by the presence of ordered regions in the matrix. Probe techniques have the potential advantage of providing molecular level information about the state of an amorphous material but because of their indirect nature require considerable care in their execution and expertise in data interpretation.

Spectroscopic techniques are valuable for the characterization of amorphous systems because of their high structural resolution. Nuclear magnetic resonance (NMR), Raman, infrared (IR) and electron spin resonance (ESR) studies of amorphous pharmaceutical systems have all been described.[57,62–64] These methods can be used to (i) determine the glass transition temperature, (ii) quantify the percent amorphous content, and (iii) determine mean molecular relaxation times as a function of temperature.[57–64] While there are some advantages in being able to follow the molecular mobility of specific chemical groups within a molecule (e.g., resolution of different modes of motion), the interpretation of spectroscopic data is often quite complex. This can mean that the conclusions drawn are open to question unless supporting evidence can be obtained using complementary techniques.[65] Samples ranging from simple powders or solutions to entire dosage forms can be studied using these nondestructive techniques, and this makes them especially useful for characterizing pharmaceutical systems. Dielectric relaxation and dynamic mechanical spectroscopy have also been widely used to study amorphous pharmaceutical materials.[66–68] These techniques are especially sensitive to the glass transition event and to secondary thermal transitions which reflect lower order molecular motions. The assignment of molecular motions measured using these techniques to particular structural groups is possible only with simple systems, and differentiation between crystalline and amorphous phases is not usually quantitative. A relatively wide frequency range can be used and activation energies for transitions can be determined. In addition, time–temperature superpositioning may be used to predict the effects of conditions that are not experimentally attainable.[69] Relatively large samples (>100 mg) are required for these two techniques, and sample preparation can sometimes be difficult.

Thermal analytical methods have been widely used to characterize amorphous pharmaceutical systems, and several comprehensive reviews of their use have been published.[70,71,72] Most often thermal analytical techniques are simply used to determine the glass transition temperature of an amorphous drug or excipient. For example, Porter and Ridgway[73] used differential scanning calorimetry (DSC) to determine the glass transition temperature of several enteric coating polymers and correlated the results with a softening point measured using a simple indentation device. The glass transition temperature
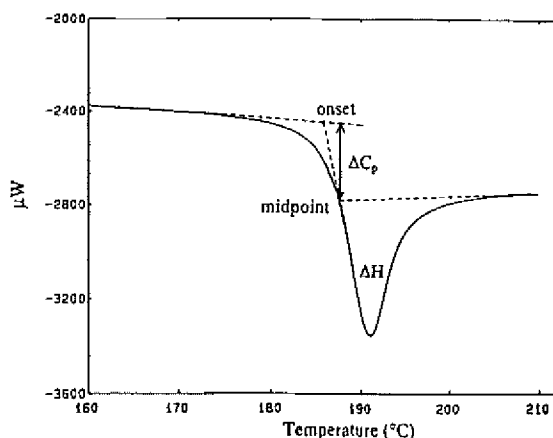
Figure 5—Glass transition of poly(vinylpyrrolidone) by DSC showing the onset and midpoint of the transition, the change in heat capacity at T_g (ΔC_p), and the enthalpy relaxation endotherm (ΔH).

of mixtures of amorphous materials (e.g., a film coating agent and a plasticizer) has also been determined as a function of mixture composition and regions of miscibility identified.[36,74,75] Thermal analytical methods can obviously be used to determine fundamental thermodynamic properties (e.g., heat capacity, enthalpy changes) of the amorphous state. The heat capacity of an amorphous material is always higher than that of its crystalline state (Figure 2), and a change in the rate of heat capacity change with temperature defines its calorimetric glass transition temperature[24] (Figures 2 and 5). This change in heat capacity at the glass transition temperature (ΔC_p) can be related to the fragility of the amorphous material (see earlier).[7,8] Glasses are thought to be frozen into an unstable state on normal experimental time scales, but on longer time scales they will slowly relax back to the metastable supercooled liquid state. The enthalpic changes associated with this process can be measured using DSC[30] and used to determine mean molecular relaxation times (τ) for glassy materials. Such relaxation processes can also be studied by thermomechanical analysis (TMA) for some sample configurations.[30,76] Crystallization from the amorphous state can be induced by thermoanalytical techniques to produce an exothermic change whose magnitude is quantitatively related to the extent of crystallization occurring. This can be used to determine the initial percent crystallinity of a partially amorphous sample provided total crystallization is known to occur.[5,41] Thermal analytical methods can also be used to study the contributions of nucleation and crystal growth processes to the overall rate of crystallization.[77] The influences of the experimental variables used in thermal analytical methods (e.g., sample size, heating rate, cooling rate) upon the results obtained with amorphous materials have been widely studied and can have very profound effects upon the type and quality of data obtained.[78] Great caution is required in the application of thermal analytical techniques to characterize the thermodynamically unstable glassy amorphous state. The variance of the glass transition temperature with heating and/or cooling rate can be used to determine the activation energy for the glass transition, which can then be used to characterize the strength/fragility of an amorphous system.[7,8] The use of more sophisticated thermal analytical techniques such as modulated DSC and thermally stimulated current methods[79,80] has great merit in the characterization of the amorphous state but is beyond the scope of this review. For a full description of these evolving techniques the reader is referred to the current thermal analysis literature.[80–82]

Vapor sorption by amorphous and crystalline pharmaceutical materials is usually quite different and thus can be used

to precisely distinguish between them.[3,27,83] Typically, crystalline materials *adsorb* vapors in small quantities at their surfaces, or take up larger stoichiometric quantities to form solvates. In contrast, amorphous materials *absorb* vapors in relatively large amounts (100% by weight is not uncommon). Vapor sorption in glassy and rubbery amorphous systems has been widely studied experimentally and modeled extensively, particularly with polymers.[84,85] The models most applicable to pharmaceutical materials are based on solution theories and assume no specific interactions between sorbent and sorbate.[86] Rubbery amorphous materials usually sorb considerably greater quantities of vapor than their respective glasses, and this phenomenon can be used to identify the state of an amorphous material.[33] Many sorbents plasticize the amorphous material into which they absorb, and thus a critical partial vapor pressure and corresponding system composition exist at which the glass to rubber transition is induced at ambient temperature.[33,40] Temperature effects on vapor sorption tend to be small, usually with an increased level of sorption with decreasing temperature which is consistent with an exothermic solution model.[86] Water vapor sorption has been most widely used in the study of amorphous drugs and excipients, and the predominant techniques are the vacuum microbalance and desiccator/saturated salt solution gravimetric methods.[33,87,88] Volumetric and potentiometric methods have also been used.[89,90] The sensitivity of water vapor sorption techniques to amorphous material is of the order of a few weight percent,[41] and thus these techniques are the preferred means of studying pharmaceutical systems containing low levels (<10%) of amorphous material. The plasticizing effects of water vapor on amorphous pharmaceutical materials have been described in some detail[33,36,83] and can potentially induce unwanted effects such as spontaneous phase transitions and lyophile collapse.[42,91] Isothermal microcalorimetric techniques can be used in conjunction with water vapor sorption methods to study such phase transitions from the amorphous state.[91] The influence of additives on the kinetics of amorphous to crystalline phase transition in lactose have been investigated,[92] as has the physical stability of amorphous drugs.[93] Typically the amorphous content of a partially crystalline material can be quantified and the enthalpies of sorption and crystallization determined.

Without water vapor sorption capabilities microcalorimetry can be used in a non-isothermal mode to detect glass transition events and secondary transitions in amorphous pharmaceutical solids. It has also been used in several studies to detect physical and chemical instabilities in formulations containing amorphous drugs or excipients.[94] Isothermal solution calorimetry has been successfully used to identify and quantify the degree of crystallinity in several pharmaceutical systems.[3] Microcalorimetry has the advantage of great thermal sensitivity, which can be very useful for studying weak secondary transitions in amorphous systems such as proteins. It has the disadvantage of being chemically unspecific, and thus the processes occurring in the calorimeter have to be independently verified using other analytical techniques. Microcalorimetric instrumentation is widely available, and many pharmaceutical research and development groups are using the technique routinely to study amorphous materials.

In summary, there are many precise and accurate methods suitable for studying and characterizing amorphous pharmaceutical materials in all their configurations, including final dosage forms. As with any scientific method, the use of multiple complimentary techniques which measure the properties most relevant to the problem or material under consideration is preferred. The most notable difference between the techniques described is in their ability to quantify the amount of order and disorder in partially amorphous systems,

Table 2—Water Content and Glass Transition Temperature of the Amorphous Fraction of a Partially Crystalline Sucrose Sample Containing Different Amounts of Water

Total Amount of Water (%)	Amorphous Content (%)	Water Content of Amorphous Fraction (g of H_2O/100 g of solid)	Glass Transition Temp (°C)
0.0	100	0	74
0.1	5.0	2	47
	2.5	4	26
	1.0	10	–17
0.5	5.0	10	–17
	2.5	20	–52
	1.0	50	–91

and in their ability to monitor molecular level processes over a wide range of temperatures (ideally above and below T_g).

Pharmaceutical Significance of the Amorphous State

Typically, the pharmaceutical scientist concerned with the use of drugs and excipients in solid dosage forms must deal with those factors that influence manufacturability, stability, bioavailability, and therapeutic performance. Although in the majority of cases drug entities come to the development stage in crystalline form, there are situations where they exist in a partially or fully amorphous form.[1] In some cases it may simply be impossible to crystallize the material by available crystallization techniques. Processing (e.g., milling, lyophilization, granulating, drying) might introduce a certain level of amorphous structure to an otherwise highly crystalline material.[27,41] The amorphous state may also be introduced deliberately to enhance the biopharmaceutical properties of the product. For example, for a crystalline drug with very poor aqueous solubility, the formation of a coamorphous mixture with a water soluble additive can provide an opportunity to enhance dissolution and perhaps bioavailability.[4,95] Additionally many excipients come to the formulator in a fully or partially amorphous state (e.g., microcrystalline cellulose, starch, poly(vinylpyrrolidone)), and other excipients may be purposefully rendered amorphous to enhance functionality (e.g., spray-dried lactose).

Since molecules in the amorphous state exist in a higher energy state than in the crystalline state, we would expect that properties requiring certain levels of molecular mobility (e.g., permeability) would be influenced by the presence of amorphous structure and by the relationship of the operating temperature to T_g and T_m. Small amounts of absorbed water can plasticize amorphous solids[36] so we would also expect relative humidity to be an important factor influencing the solid-state properties of amorphous systems. It has been shown that, if a system consists of a small amount of amorphous material in a crystalline matrix, the preferential absorption of water vapor into the amorphous structure will result in a local water content greatly amplified relative to the total water content, with a correspondingly greater plasticizing effect on the amorphous structure[27] (Table 2).

In considering some examples of the importance of the amorphous state in determining the solid-state properties of pharmaceuticals, we will examine three broad areas: crystallization, chemical degradation, and mechanical responses to stress (e.g., powder compression in tabletting). In all of these cases a critical factor is the rate at which molecules in the solid state undergo rotational or translational motions. As discussed previously (see eqs 1 and 2) molecular mobility changes significantly with temperature, additive concentration, and water content, particularly in the vicinity of T_g. The

ultimate challenge is to relate the time scales required for various physical and chemical solid-state processes to the time scales expected for molecular mobility under any given set of environmental conditions. A few illustrations of the state of our knowledge in applying these concepts to pharmaceutical systems are given below.

Crystallization—Since molecules in the amorphous state are thermodynamically metastable relative to the crystalline state, the potential for crystallization during handling and storage is always present. Evidence exists to show that such crystallization can be responsible for phenomena such as postcompression hardening of tablets,[96,97] lyophilized cake collapse,[98] and particle aggregation in dry powder inhalers.[99] It would appear important to be able to anticipate conditions that give rise to such crystallization and to be able to inhibit it if so desired.

Crystallization from the amorphous state is primarily governed by the same factors that determine crystallization from the melt. Hence we can illustrate the important factors to be considered by observing the general equations for homogeneous nucleation from the melt.[100] The rate of nucleation I is expressed as

$$I = A \exp \frac{-(\Delta G_a + \Delta G^*)}{kT} \tag{6}$$

where ΔG_a = the activation energy for transport across the nucleus–amorphous matrix interface, ΔG^* = the free energy change for nucleation with a critical nucleus of radius r, A is a constant, k is the Boltzmann constant, and T is the temperature, and where for a spherical nucleus it can be shown that

$$\Delta G^* = \frac{16\Pi \Delta g^3}{3\Delta G_v^3} \tag{7}$$

where ΔG_v = the difference in free energy per unit volume between the crystalline and amorphous forms, $G_c - G_L$, and Δg = the interfacial free energy per unit area at the nucleus–amorphous matrix interface. Further, it can be shown that

$$\Delta G_v = (G_c - G_L) \approx -(\Delta H_f)(\Delta T_r) \tag{8}$$

where ΔH_f = heat of fusion, $\Delta T_r = (1 - T_c/T_m)$ = degree of supercooling, T_m = melting temperature, and T_c = crystallization temperature.

From these equations we can see that for any amorphous system the temperature at which optimal nucleation, and hence crystallization, should occur will depend primarily on the degree of supercooling below T_m and where the temperature lies relative to T_g. The closer to T_g, the greater the degree of supercooling and the lower the molecular mobility. As described by Jolley[101] and schematically shown in Figure 6, the enhanced tendency for supercooling caused by lowering the temperature and increased viscosity that accompanies this reduction in temperature should give rise to a maximal crystallization rate at some temperature between T_g and T_m. Indeed a non-isothermal DSC scan of amorphous sucrose (Figure 7) shows a crystallization exotherm somewhere between the T_g and T_m.[41] It also has been shown that the crystallization temperature for amorphous sugars is reduced by the presence of absorbed water in direct proportion to the plasticizing effects of water on T_g and raised by the incorporation of high T_g additives, again demonstrating the importance of molecular mobility in this process.[28] This has led to a general conclusion that crystallization from the amorphous state over practical time scales can be prevented by keeping the operating temperature below T_g.[101,102] by reducing the
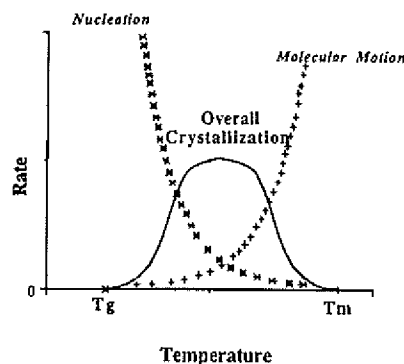
Figure 6—Schematic depiction of the parameters controlling crystallization from the amorphous state. Adapted from ref 101.
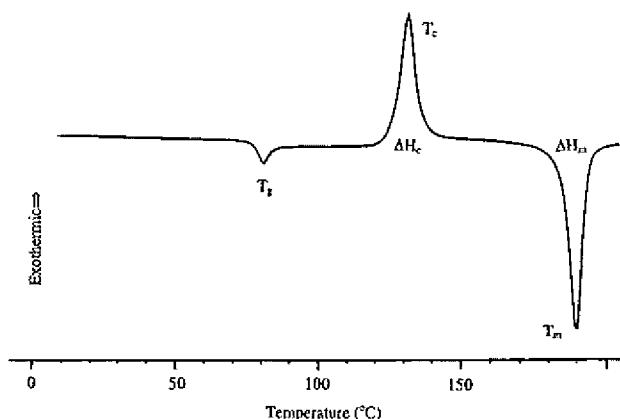

Figure 7—DSC scan of amorphous sucrose showing the glass transition temperature (T_g), the crystallization exotherm $(T_c, \Delta H_c)$, and the melting endotherm $(T_m, \Delta H_m)$. Adapted from refs 41 and 42.

water content or by raising the T_g of the system using additives with high T_g values[103] (*e.g.*, PVP with a T_g of about 180 °C[33]). If a small amount of amorphous structure is introduced into a crystalline sample by processing[3,4,104] and this is deemed to be undesirable, raising the operating temperature above T_g, or exposing the sample to elevated relative humidity so as to lower T_g, can "anneal" the sample back to a completely crystalline state.

An important question with regard to crystallization is, *At how low a temperature must an amorphous sample be stored to provide typical 3–5 year shelf-life stability?* In recent studies with dry amorphous indomethacin having a T_g of about 50 °C it was shown that complete crystallization occurred within a few weeks at temperatures as low as 20 °C, but that reducing the temperature to refrigerator temperature provided periods of stability of a year or more.[5] An analysis of relaxation times for indomethacin below its T_g, using enthalpic relaxation measurements[30] and a fit of these data to the VTF equation (see eq 1) revealed a value of T_o equal to about 267 °C. Assuming that this corresponds to the temperature at which essentially all important molecular motions would cease,[102] it was concluded that storage in a freezer (<267 °C) should provide at least 3–5 year shelf-life stability. An alternative means of stabilizing an amorphous drug would have been to raise its T_g by cosolidifying with a high-T_g glass former such as PVP.[103] These strategies have been advocated by many workers, but a clear understanding of the molecular basis for such stabilizing effects has not yet been achieved.[105–109] More work of this type is still required, and for further information the interested reader is referred to some general reviews related to crystallization from the amorphous state.[5,27,28,41,110–112]

Chemical Reactivity—Chemical degradation of drugs in the solid state, particularly at elevated temperatures and relative humidities, is a fairly common occurrence with drugs exhibiting a susceptibility for degradation when in solution. Presumably, in the amorphous state the reacting molecules have sufficient free volume and molecular mobility to react. We would expect a comparison of reaction rates of crystalline and amorphous forms of a drug under otherwise identical conditions to reveal greater rates with the amorphous forms than with the crystalline forms, and this has been shown to be the case for a number of systems.[113,114] It is possible that a particularly solid state reaction would require a high level of positional specificity between reacting species and that this would occur most readily in a highly ordered crystalline state. In such cases reactivity in the amorphous or liquid states would be reduced relative to that in the crystalline state.[115,116] In the case of insulin it has been reported that some degradation pathways occur more readily in the amorphous state while in one particular reaction requiring such specific positioning the crystalline state is more reactive.[117]

If enhanced molecular mobility in the amorphous state is the major factor in controlling reaction rates, we would expect dependence of reactivity on free volume resulting in non-Arrhenius kinetics.[118] Thus an equation that combines activational energetics with free volume considerations has been derived:[119]

$$k = A' \exp\left[-\left(\frac{V^*}{V_f}\right)-\left(\frac{E_a}{kT}\right)\right] \tag{9}$$

where k is the rate constant, A' a pre-exponential factor, E_a is the energy of activation, V_f is the free volume, and V^* is the critical free volume for the molecular motion required for reactivity. For systems with high V_f (well above T_g or in the liquid state) the rate constant should follow Arrhenius kinetics, while at lower temperatures closer to and below T_g free volume should be an important factor. As an example of the first case we can consider a recent study of degradation of indomethacin in the liquid state (above T_m) and just below T_m in the amorphous state (about 100 °C above T_g), where it was shown that the rate constants fit the Arrhenius equation with no discontinuity as the sample changed from the amorphous solid to the melt.[113] If free volume factors are important, we would expect the non-Arrhenius behavior to parallel the effects of temperature on relaxation rates, as reflected in the VTF or WLF equations described earlier (eqs 1 and 2). Depending on the fragility of the amorphous state we might expect greater or lesser deviations from Arrhenius behavior. The situation is complicated by the fact that any system studied over a limited temperature range will exhibit apparent linearity on an Arrhenius plot. Consequently, to test for temperature dependence in such amorphous systems it is best to use as wide a temperature range as possible and to work under conditions in the vicinity of T_g where free volume effects change significantly. Such a situation seems to exist in the case of the nonenzymatic browning reaction that typically occurs between reducing sugars and amines where it has been shown that reaction rates correlate with $T - T_g$ (as in eqs 1 and 2) in a number of cases.[120] To date in the pharmaceutical literature there has been only one published study of an amorphous system that appears to follow WLF-like chemical kinetics. Roy *et al.*[121] measured the rates of three different types of chemical degradation for lyophilized samples of a *Vinca* alkaloid–antibody conjugate and showed excellent correlation of reaction rates with $T - T_g$ for two temperatures and three water contents (different values of T_g). It is important to recognize that some reactions in the amorphous state can still take place below T_g and that

attempts to scale reaction kinetics to T_g may be inappropriate. In such cases the value of T_0 in the VTF equation (see eq 1) may be more physically meaningful. More studies covering a wide range of temperatures and using other models are required to address this important issue if we ultimately expect to be able to predict chemical kinetic behavior in such systems.

Mechanical Properties—In the processing and handling of solid pharmaceuticals there are a number of situations where rheological or mechanical properties are critical for product manufacturability, stability, and performance. Typically most crystalline materials tend to exhibit high levels of elasticity and brittleness upon exposure to an external stress. By contrast molecules in the amorphous state tend to exhibit varying degrees of viscoelasticity, depending on their temperature relative to T_g. Such viscoelastic behavior provides solids with the ability to flow under conditions of mechanical stress and to provide a number of important excipient functionalities. Relief of mechanical stresses through flow would appear to be important in creating tablet bonds after compression of powders[97,122] and in preventing mechanical failure of polymeric film coats on tablets because of stress relaxation.[123] The use of plasticizers, including water, to lower the T_g of various cellulosic and acrylic polymers utilized in film coating is based on this principle, as is the use of spray-dried lactose (about 15% amorphous content) or microcrystalline cellulose (about 30% amorphous content) as direct compression tabletting excipients. To emphasize the importance of the amorphous state in these latter situations it should be pointed out that the water content of such direct compression excipients must be held at a certain level to provide optimal compression properties. For example, a critical level of about 4—6% water provides a significant change in the viscoelastic properties of microcrystalline cellulose[124] and is the level below which direct compact properties are lost. Similarly spray drying crystalline sucrose with 3% maltodextrin produces a direct compression material with properties strongly dependent on the water content of the maltodextrin[125,126] presumably because of its plasticizing effects on the amorphous component.

Strategies for Dealing with Partially Amorphous Pharmaceutical Systems

Let us consider a new drug entity or excipient that contains irregular particles which are not birefringent under cross-polarized light. The material also exhibits nonstoichiometric water uptake in excess of that expected for surface adsorption on a crystalline surface (e.g., >0.01%)[83,104] and upon exposure to elevated temperature and relative humidity appears to undergo chemical degradation in a manner similar to its behavior in solution or in the melt. Such characteristics should immediately suggest that the material may have considerable amorphous character.[41,83] At this point it would be useful to check on the history of the processes used to prepare the material: How was it crystallized? Did it undergo any milling that might have led to the creation of amorphous character? Were there any additives present or processing conditions that might stabilize or favor the amorphous state? To further assess the characteristics of this system we can take two approaches. First, we can attempt to predict the properties of an amorphous state based on characteristics of the 'crystalline' sample; and second, we can attempt to prepare a completely amorphous form of the drug and carry out direct measurements of its properties.

In the first case we can begin by measuring the crystal melting temperature (T_m). This can then be used to estimate the glass transition temperature based on the fact that the mean ratio of the melting temperature to the glass transition

temperature (T_m/T_g) in kelvin for pharmaceutical materials is approximately 1.36[70,75] (Table 1). An estimate of the Kauzmann temperature (T_K) (or the point of 'negligible' molecular mobility) can also be made as $T_m/2$ or $T_g -50$ K. The heat capacity change at T_g (ΔC_p) can be estimated from the presumably known chemical structure[127–129] or from the knowledge that for many materials (especially polymers) the product of ΔC_p and T_g is approximately 100 kJ·g^{-1}. Likewise the product of T_g and the change in thermal expansivity at T_g ($\Delta\alpha$) is approximately 0.113 leading to an estimate for $\Delta\alpha$.[130,131] The viscosity of most glasses is between 10^9 and 10^{13} Pa·s at T_g and above T_g often varies with temperature according to the WLF equation (with so-called universal constants) so a preliminary estimate of molecular mobility at any temperature above T_g could be made. A prediction of the fragility of the amorphous material also could be made from the estimated ΔC_p and T_m/T_g values,[7,8,132] and from this knowledge a more accurate assessment of the temperature dependence of molecular mobility and viscosity above T_g could be attempted.[133] Assuming ideal behavior, the plasticizing effect of any absorbed water vapor (or other additive/penetrant) on the amorphous material can be estimated using simple mixing rules.[36] This should allow the determination of the critical plasticizer content at which the glass to rubber transition is induced at ambient or other operating temperature. If it is possible to obtain a purely amorphous sample, direct characterization of this form can then be carried out. Amorphous reference samples can usually be produced by either quench-cooling the melt in liquid nitrogen, lyophilization, or spray drying. Other than powder X-ray diffraction measurements for assessing lack of crystallinity, the most useful techniques for the early evaluation of amorphous solids are differential scanning calorimetry and water vapor sorption analysis. A non-isothermal DSC scan of a dry amorphous material can be used to measure T_g, ΔC_p, T_c (non-isothermal recrystallization temperature), and T_m (Figure 6). From these parameters characterization of the amorphous material can be carried out in a reasonable level of detail. Questions that can be answered include the following: What is the glass transition temperature relative to the ambient temperature? What is T_g relative to T_m, and thus what is the fragility of the compound? Does the amorphous material spontaneously crystallize upon heating above T_g, and if so, at what temperature? Can crystallization be induced by scratching the surface of the sample, or by exposing to water vapor? All of these questions may be addressed using only a small quantity (a few milligrams) of material. The answers to these questions can be compared to those predicted using the "rules of thumb" given earlier and any deviations from typical amorphous behavior identified. Water vapor sorption by the amorphous material should be measured next at room temperature over a wide range of relative humidities. Since it has been shown that water vapor absorption varies very little over the temperature range 5—60 °C for many systems,[86] this data can be taken to be representative of water uptake at most temperatures experienced by a pharmaceutical solid. The use of an automated sorption microbalance apparatus[87] should allow such measurements to be performed with only a few milligrams of material. If water absorbed into the solid is able to cause crystallization over a short time scale, this will be reflected by a spontaneous loss of the absorbed water at some critical relative humidity. This will give an indication of what water content (or humidity) will be required to eliminate the amorphous content of any sample, or below what water content one must store amorphous samples to avoid crystallization. These experiments can be followed by more precise measurements of T_g vs water content by DSC to provide a more exact indication of how water might act as a plasticizer and what water content and relative humidity are required

to induce the glass to rubber transition at ambient temperature. Comparisons with the behavior predicted by simple mixing rules could also be made in order to obtain an understanding of the nature of amorphous solid–water interactions.[36,37,39] Thus, from a few simple calculations and measurements, it is possible to determine many of the physical properties of the amorphous form of a new material, and to assess its likely behavior in a pharmaceutical dosage form. Further detailed physicochemical characterization with the techniques described earlier will obviously be desirable, as will accelerated stability tests and biopharmaceutical studies.

Conclusions

The amorphous state is critical in determining the behavior and properties of many pharmaceutical formulations. Through an appreciation of the properties of the amorphous state it is possible to define means with which to characterize and work with such molecularly disordered pharmaceutical systems. In cases where amorphous character is desirable in a pharmaceutical formulation, it may be stabilized using strategies based on an understanding of the thermodynamic and kinetic properties of amorphous systems. In circumstances where amorphous character is undesirable, approaches are available so that disorder can be minimized and conversion of amorphous material to the most stable crystalline state can be promoted.

Note Added in Proof

While this manuscript was in press, it was pointed out that another reference predates refs 7 and 8 in reference to the rule of thumb proposed for determining fragility: Slade, L.; Levine, H. *Adv. Food Nutr. Res.* **1995**, *38*, 103–269.

References and Notes

1. Byrn, S. R. *Solid state chemistry of drugs;* Academic Press: New York, 1982.
2. Roy, R. *J. Non-Cryst. Solids* **1970**, *3*, 33–40.
3. Pikal, M.; Lukes, A.; Lang, J.; Gaines, K. *J. Pharm. Sci.* **1978**, *67*, 767–773.
4. Huttenrauch, R. *Acta Pharm. Technol., Suppl.* **1978**, *6*, 55–127.
5. Yoshioka, M.; Hancock, B. C.; Zografi, G. *J. Pharm. Sci.* **1994**, *83*, 1700–1705.
6. Mansfield, M. *J. Chem. Phys.* **1995**, *103*, 8124–8129.
7. Angell, C. A. *Proc. Nat. Acad. Sci., U.S.A.* **1995**, *92*, 6675–6682.
8. Angell, C. A. *Science* **1995**, *267*, 1924–1935.
9. Angell, C. A.; MacFarlane, D. R.; Oguni, M. *Ann. N.Y. Acad. Sci.* **1986**, *484*, 241–247.
10. Ediger, M. D.; Angell, C. A.; Nagel, S. R. *J. Phys. Chem.* **1996**, *100*, 13200–13212.
11. Mansfield, M. L. In *The glassy state in foods;* Blanshard, J. M. V., Lillford, P. J., Eds.; Nottingham University Press: Loughborough, 1993; pp 103–122.
12. McKenna, G. B. In *Comprehensive Polymer Science;* Booth, C., Price, C., Eds.; Pergamon Press: New York, 1989; pp 311–362.
13. Koike, T. *J. Appl. Polym. Sci.* **1995**, *56*, 1183–1186.
14. Mayes, A. M. *Macromolecules* **1994**, *27*, 3114–3115.
15. Keddie, J. L.; Jones, R. A. L.; Cory, R. A. *Faraday Discuss.* **1994**, *98*, 219–230.
16. Her, L.-M.; Deras, M.; Nail, S. L. *Pharm. Res.* **1995**, *12*, 768–772.
17. Williams, M. L.; Landel, R. F.; Ferry, J. D. *J. Am. Chem. Soc.* **1955**, *77*, 3701–3707.
18. Ollett, A.-L.; Parker, R. *J. Texture Stud.* **1990**, *21*, 355–362.
19. Parks, G. S.; Barton, L. E.; Spaght, M. E.; Richardson, J. W. *Physics* **1934**, *5*, 193–199.
20. Soesanto, T.; Williams, M. C. *J. Phys. Chem.* **1981**, *85*, 3338–3341.
21. Green, P. F. *J. Non-Cryst. Solids* **1994**, *172–174*, 815–822.
22. Matsuda, Y.; Otsuka, M.; Onoe, M.; Tatsumi, E. *J. Pharm. Pharmacol.* **1992**, *44*, 627–633.
23. Hachisuka, H.; Takizawa, H.; Tsujita, Y.; Takizawa, A.; Kinoshita, T. *Polymer* **1991**, *32*, 2382–2386.
24. Tsukushi, I.; Yamamuro, O.; Suga, H. *J. Non-Cryst. Solids* **1994**, *175*, 187–194.
25. Guo, J. H.; Robertson, R. E.; Amidon, G. L. *Pharm. Res.* **1991**, *8*, 1500–1504.
26. Byron, P. R.; Dalby, R. N. *J. Pharm. Sci.* **1987**, *76*, 65–67.
27. Ahlneck, C.; Zografi, G. *Int. J. Pharm.* **1990**, *62*, 87–95.
28. Saleki-Gerhardt, A. Role of water in the solid-state properties of crystalline and amorphous sugars. Ph.D. Thesis, University of Wisconsin–Madison, 1993.
29. Hodge, I. M. *Science* **1995**, *267*, 1945–1947.
30. Hancock, B. C.; Shamblin, S. L.; Zografi, G. *Pharm. Res.* **1995**, *12*, 799–806.
31. Mishima, O. *Science* **1991**, *254*, 406–408.
32. Williams, E.; Angell, C. A. *J. Phys. Chem.* **1977**, *81*, 232–237.
33. Oksanen, C. A.; Zografi, G. *Pharm. Res.* **1990**, *7*, 654–657.
34. Angell, C. A.; Bressel, R. D.; Green, J. L.; Kanno, H.; Oguni, M.; Sare, E. J. *J. Food. Eng.* **1994**, *22*, 115–142.
35. Gordon, M.; Taylor, J. S. *J. Appl. Chem.* **1952**, *2*, 493–500.
36. Hancock, B. C.; Zografi, G. *Pharm. Res.* **1994**, *11*, 471–477.
37. Schneider, H. A. *Polymer* **1989**, *30*, 771–779.
38. Aubin, M.; Prud'homme, R. E. *Macromolecules* **1988**, *21*, 2945–2949.
39. Shamblin, S.; Huang, E. Y.; Zografi, G. *J. Therm. Anal.*, in press.
40. Lewis, A. F.; Tobin, M. C. *J. Appl. Polym. Sci.* **1962**, *6*, S24–25.
41. Saleki-Gerhardt, A.; Ahlneck, C.; Zografi, G. *Int. J. Pharm.* **1994**, *101*, 237–247.
42. Saleki-Gerhardt, A.; Zografi, G. *Pharm. Res.* **1994**, *11*, 1166–1173.
43. Timko, R. J.; Lordi, N. G. *Drug. Dev. Ind. Pharm.* **1984**, *10*, 425–451.
44. Fecht, H. J. *Mater. Trans., JIM* **1995**, *36*, 777–793.
45. Suga, H. *Thermochim. Acta* **1994**, *245*, 69–81.
46. Clas, S.-D.; Faizer, R.; O'Connor, R. E.; Vadas, E. B. *Int. J. Pharm.* **1995**, *121*, 73–79.
47. Roe, R.-J.; Curro, J. J. *Macromolecules* **1983**, *16*, 428–434.
48. Frick, B.; Richter, D. *Science* **1995**, *267*, 1939–1944.
49. Nara, S. *Starch* **1979**, *31*, 73–75.
50. Duncan-Hewitt, W. C.; Grant, D. J. W. *Int. J. Pharm.* **1986**, *28*, 75–84.
51. Richardson, M. J.; Savill, N. G. *Polymer* **1977**, *18*, 3–9.
52. Tarjus, G.; Kivelson, D. *J. Chem. Phys.* **1995**, *103*, 3071–3073.
53. Stillinger, F. H. *Science* **1995**, *267*, 1935–1939.
54. Kolde, M.; Sato, R.; Komatsu, T.; Matusita, K. *Phys. Chem. Glasses* **1995**, *36*, 172–175.
55. Vrentas, J. S.; Vrentas, C. M. *Macromolecules* **1994**, *27*, 5570–5576.
56. Vrentas, J. S.; Vrentas, C. M. *Macromolecules* **1994**, *27*, 4684–4690.
57. Oksanen, C. A.; Zografi, G. *Pharm. Res.* **1993**, *10*, 791–799.
58. Han, J.; Gee, R. H.; Boyd, R. H. *Macromolecules* **1994**, *27*, 7781–7784.
59. Shah, N. K.; Ludescher, R. D. *Biotechnol Prog.* **1995**, *11*, 540–544.
60. Roozen, M. J. G. W. *J. Phys. Chem.* **1990**, *94*, 7326–7329.
61. Roozen, M. J. G. W.; Hemminga, M. A.; Walstra, P. *Carbohydr. Res.* **1991**, *215*, 229–237.
62. Glotin, M.; Mandelkern, L. *Colloid Polym. Sci.* **1982**, *260*, 182–192.
63. Yoshimura, Y.; Kanno, H. *J. Solution Chem.* **1995**, *24*, 633–639.
64. Strobl, G. R.; Hagedorn, W. *J. Polym. Sci., Polym. Phys. Ed.* **1978**, *16*, 1181–1193.
65. Srcic, S.; Kerc, J.; Urleb, U.; Zupancic, I.; Lahajnar, G.; Kofler, B.; Smid-Korbar, J. *Int. J. Pharm.* **1992**, *87*, 1–10.
66. Craig, D. Q. M. *Drug. Dev. Ind. Pharm.* **1992**, *18*, 1207–1223.
67. Craig, D. Q. M.; Johnson, F. A. *Thermochim. Acta* **1995**, *248*, 97–115.
68. Kararli, T. T.; Hurlbut, J. B.; Needham, T. E. *J. Pharm. Sci.* **1990**, *79*, 845–848.
69. Ferry, J. D. *Viscoelastic properties of polymers*, 3rd ed.; John Wiley: New York, 1980.
70. Kerc, J.; Srcic, S. *Thermochim. Acta* **1995**, *248*, 81–95.
71. Giron, D. *Acta Pharm. Jugosl.* **1990**, *40*, 95–157.
72. Giron, D. *Thermochim. Acta* **1995**, *248*, 1–59.
73. Porter, S. C.; Ridgway, K. *J. Pharm. Pharmacol.* **1983**, *35*, 341–344.
74. Sakellariou, P.; Rowe, R. C. *J. Macromol. Sci., Pure Appl. Chem.* **1994**, *A31*, 1201–1208.
75. Fukuoka, E.; Makita, M.; Yamamura, S. *Chem. Pharm. Bull.* **1989**, *37*, 1047–1050.
76. Fukuoka, E.; Makita, M.; Nakamura, Y. *Chem. Pharm. Bull.* **1989**, *37*, 2782–2785.
77. Angell, C. A.; Alba, C.; Arzimangolou, A.; Boehmer, R.; Fan, J.; Lu, Q.; Sanchez, E.; Senapati, H.; Tatsumisago, M. *AIP. Conf. Proc.* **1992**, *256*, 3–19.

78. Moynihan, C. T.; Easteal, A. J.; Wilder, J.; Tucker, J. *J. Phys. Chem.* **1974**, *78*, 2673–2677.
79. Tomasi, C.; Mustarelli, P.; Hawkins, N. A.; Hill, V. *Thermochim. Acta* **1996**, *278*, 9–18.
80. Boller, A.; Jin, Y.; Wunderlich, B. *J. Therm. Anal.* **1994**, *42*, 307–330.
81. Coleman, N. J.; Craig, D. Q. M. *Int. J. Pharm.* **1996**, *135*, 13–29.
82. Sauer, B. B.; Avakian, P. *Polymer* **1992**, *33*, 5128–5142.
83. Zografi, G.; Hancock, B. C. In *Topics in Pharmaceutical Sciences 1993, Proceedings of International Congress on Pharmaceutical Sciences F.I.P.*; Crommelin, D. J. A., Midha, K. K., Nagai, T., Eds; Medpharm Scientific: Stuttgart, Germany, 1994; pp 405–419.
84. Vrentas, J. S.; Vrentas, C. M. *Macromolecules* **1991**, *24*, 2404–2412.
85. Lipscomb, G. G. *AIChE J.* **1990**, *36*, 1505–1516.
86. Hancock, B. C.; Zografi, G. *Pharm. Res.* **1993**, *10*, 1262–1267.
87. Bergren, M. S. *Int. J. Pharm.* **1994**, *103*, 103–114.
88. Callahan, J.; Cleary, G.; Elefant, M.; Kaplan, G.; Kensler, T.; Nash, R. *Drug Dev. Ind. Pharm.* **1982**, *8*, 355–369.
89. Kontny, M. J. Water vapor sorption studies on solid surfaces. Ph.D. Thesis, University of Wisconsin—Madison, Madison, WI, 1985.
90. Spitz, H. *J. Pharm. Sci.* **1979**, *68*, 122.
91. Angberg, M.; Nystrom, C.; Castensson, S. *Int. J. Pharm.* **1992**, *83*, 11–23.
92. Buckton, G.; Darcy, P. *Int. J. Pharm.* **1995**, *121*, 81–87.
93. Aso, Y.; Yoshioka, S.; Otsuka, T.; Kojima, S. *Chem. Pharm. Bull.* **1995**, *43*, 300–303.
94. Heidemann, D. R.; Jarosz, P. J. *Pharm. Res.* **1991**, *8*, 292–297.
95. Simonelli, A. P.; Mehta, S. C.; Higuchi, W. I. *J. Pharm. Sci.* **1976**, *65*, 335–361.
96. Lordi, N.; Shiromani, P. *Drug. Dev. Ind. Pharm.* **1984**, *10*, 729–752.
97. Elamin, A. A.; Alderborn, G.; Ahlneck, C. *Int. J. Pharm.* **1994**, *108*, 213–224.
98. Levine, H.; Slade, L. *J. Chem. Soc., Faraday Trans.* **1988**, *84*, 2619–2633.
99. Ward, G. H.; Schultz, R. K. *Pharm. Res.* **1995**, *12*, 773–779.
100. Turnbull, D.; Fisher, J. C. *J. Chem. Phys.* **1949**, *17*, 71–73.
101. Jolley, J. E. *Photogr. Sci. Eng.*, **1970**, *14*, 169–177.
102. Okamoto, N.; Oguni, M. *Solid State Commun.* **196**, *99*, 53–56.
103. Yoshioka, M.; Hancock, B. C.; Zografi, G. *J. Pharm. Sci.* **1995**, *84*, 983–986.
104. Hancock, B. C.; Zografi, G. *J. Pharm. Sci.* **1996**, *85*, 246–248.
105. Carpenter, J. F.; Crowe, J. H. *Cryobiology* **1988**, *25*, 244–255.
106. Carpenter, J. F.; Pretrelski, J.; Anchorodoguy, T. J.; Arakawa, T. *ACS Symp. Ser.* **1994**, *567*, 134–147.
107. Crowe, J. H.; Crowe, L. M.; Carpenter, J. F. *BioPharm* **1993**, *May*, 40–43.
108. Crowe, J. H.; Crowe, L. M.; Carpenter, J. F. *BioPharm* **1993**, *April*, 26–34.
109. Franks, F.; Aldous, B.; Auffret, T. *Genet. Eng. News* **1995**, *August*.
110. Mandelkern, L. *Crystallization of polymers;* McGraw-Hill: New York, 1964.
111. Hammel, J. J. In *Nucleation;* Zettlemoyer, A. C., Ed.; Marcel Dekker: New York, 1969; pp 489–525.
112. Shi, F. G. *J. Appl. Phys.* **1994**, *76*, 5149–5153.
113. Carstensen, J. T.; Morris, T. *J. Pharm. Sci.* **1993**, *82*, 657–659.
114. Oberholtzer, E. R.; Brenner, G. S. *J. Pharm. Sci.* **1979**, *68*, 863–867.
115. Sukenik, C. N.; Bonapace, J. A. P.; Mandel, N. S.; Lau, P.-Y.; Wood, G.; Bergman, R. G. *J. Am. Chem. Soc.* **1977**, *99*, 851–858.
116. Sukenik, C. N.; Bonapace, J. A. P.; Mandel, N. S.; Bergman, R. G.; Lau, P.-Y.; Wood, G. *J. Am. Chem. Soc.* **1975**, *97*, 5290–5291.
117. Pikal, M. In *Peptide and protein drug delivery;* Lee, V. H., Ed.; Marcel Dekker: New York, 1996.
118. Cohen, M. H.; Turnbull, D. *J. Chem. Phys.* **1959**, *31*, 1164–1169.
119. Macedo, P. B.; Litovitz, T. A. *J. Chem. Phys.* **1965**, *42*, 245–256.
120. Buera, M. P.; Karel, M. *Food Chem.* **1995**, *52*, 167–173.
121. Roy, M. L.; Pikal, M. J.; Rickard, E. C.; Maloney, A. M. *Dev. Biol. Stand.*, **1992**, *74*, 323–340.
122. Sebhatu, T.; Elamin, A. A.; Ahlneck, C. *Pharm. Res.* **1994**, *11*, 1233–1238.
123. Sinko, C. M. An investigation into the relaxation behaviour of pharmaceutical film coatings. Ph.D. Thesis, University of Michigan, Ann Arbor, MI, 1989.
124. Amidon, G. E.; Houghton, M. E. *Pharm. Res.* **1995**, *12*, 923–929.
125. Li, L. C.; Peck, G. E. *J. Pharm. Pharmacol.* **1990**, *42*, 272–275.
126. Tabibi, S. E. Water vapor adsorption by compressible sugar and its effect on powder compressibility. Ph.D. Thesis, University of Maryland, Baltimore, MD, 1982.
127. Van Krevelen, D. W.; Hoftyzer, P. J. *Properties of polymers: their estimation and correlation with chemical structure*, 2nd ed.; Elsevier: Amsterdam, 1976.
128. Shaw, R. *J. Chem. Eng. Data* **1969**, *14*, 461–465.
129. Hatakeyama, T.; Hatakeyama, H. *Thermochim. Acta* **1995**, *267*, 249–257.
130. Boyer, R. F. *J. Macromol. Sci. Phys.* **1973**, *B7*, 487–501.
131. Simha, R.; Boyer, R. F. *J. Chem. Phys.* **1962**, *37*, 1003–1007.
132. Angell, C. A.; Sare, J. M.; Sare, E. J. *J. Phys. Chem.* **1978**, *82*, 2622–2629.
133. Senapati, U.; Varshneya, A. K. *J. Non-Cryst. Solids* **1996**, *197*, 210–218.
134. Hofer, K.; Astl, G.; Mayer, E.; Johari, G. P. *J. Phys. Chem.* **1991**, *95*, 10777–10781.

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